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# CONTENTS.

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	PAGE
THE INFLUENCE OF BORAX AND BORIC ACID UPON NUTRITION, WITH SPECIAL REFERENCE TO PROTEID METABOLISM. <i>By R. H. Chittenden and William J. Gies</i> . . . . .	I
VARIATIONS IN DAILY ACTIVITY PRODUCED BY ALCOHOL AND BY CHANGES IN BAROMETRIC PRESSURE AND DIET, WITH A DESCRIPTION OF RECORDING METHODS. <i>By Colin C. Stewart, A.B., Toronto</i> . . .	40
THE INFLUENCE OF HIGH ARTERIAL PRESSURES UPON THE BLOOD-FLOW THROUGH THE BRAIN. <i>By W. H. Howell</i> . . . . .	57
THE RECOVERY OF THE HEART FROM FIBRILLARY CONTRACTIONS. <i>By W. T. Porter</i> . . . . .	71
NOTES ON THE ELIMINATION OF STRONTIUM. <i>By Horatio C. Wood, Jr., M.D.</i> . . . .	83
THE NUTRITION OF THE HEART THROUGH THE VESSELS OF THEBESIUS AND THE CORONARY VEINS. <i>By F. H. Pratt</i> . . . . .	86
ON THE RELATION BETWEEN THE EXTERNAL STIMULUS APPLIED TO A NERVE AND THE RESULTING NERVE IMPULSE AS MEASURED BY THE ACTION CURRENT. <i>By C. W. Greene</i> . . . . .	104
ON THE NATURE OF THE CARDIOPNEUMATIC MOVEMENTS. <i>By S. J. Meltzer</i>	117
THE FUNCTIONS OF THE EAR AND THE LATERAL LINE IN FISHES. <i>By Frederic S. Lee</i> . . . . .	128
THE INFLUENCE OF THE HEART-BEAT ON THE FLOW OF BLOOD THROUGH THE WALLS OF THE HEART. <i>By W. T. Porter</i> . . . . .	145
A FURTHER STUDY OF THE INFLUENCE OF ALCOHOL AND ALCOHOLIC DRINKS UPON DIGESTION, WITH SPECIAL REFERENCE TO SECRE- TION. <i>By R. H. Chittenden, Lafayette B. Mendel, and Holmes C. Jackson</i> . . . . .	164
ON THE SIMILARITY OF STRUCTURAL CHANGES PRODUCED BY LACK OF OXYGEN AND CERTAIN POISONS. <i>By Sidney P. Budgett</i> . . . . .	210
THE EFFECT OF DISTENTION OF THE VENTRICLE ON THE FLOW OF BLOOD THROUGH THE WALLS OF THE HEART. <i>By Ida H. Hyde, Ph.D.</i> . . . .	215
THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF SOME EDIBLE AMERICAN FUNGI <i>By Lafayette B. Mendel</i> . . . . .	225
THE RESTORATION OF COÖRDINATED VOLITIONAL MOVEMENT AFTER NERVE-"CROSSING." <i>By R. H. Cunningham, M.D.</i> . . . .	239

PAPAIN-PROTEOLYSIS, WITH SOME OBSERVATIONS ON THE PHYSIOLOGICAL ACTION OF THE PRODUCTS FORMED. <i>By R. H. Chittenden, Lafayette B. Mendel, and H. E. McDermott</i> . . . . .	PAGE 255
THE GASTRIC INVERSION OF CANE-SUGAR BY HYDROCHLORIC ACID. <i>By S. J. Ferris and Graham Lusk</i> . . . . .	277
ON THE MEASUREMENT OF MENTAL ACTIVITY THROUGH MUSCULAR ACTIVITY AND THE DETERMINATION OF A CONSTANT OF ATTENTION. <i>By Jeannette C. Welch</i> . . . . .	283
THE INFLUENCE OF BILE AND BILE SALTS ON PANCREATIC PROTEOLYSIS. <i>By R. H. Chittenden and Alice H. Albro, B.A.</i> . . . .	307
THE REINFORCEMENT OF VOLUNTARY MUSCULAR CONTRACTIONS. <i>By Allen Cleghorn, M.D.</i> . . . .	336
ON CERTAIN CHARACTERISTICS OF THE PRESSURE SENSATIONS OF THE HUMAN SKIN. <i>By Gaylord P. Clark, M.D.</i> . . . .	346
THE MOVEMENTS OF THE STOMACH STUDIED BY MEANS OF THE RÖNTGEN RAYS. <i>By W. B. Cannon</i> . . . . .	359
CONTRIBUTIONS TO THE PHYSIOLOGY OF THE CARDIAC NERVES IN THE GUINEA-PIG. <i>By D. W. Harrington, A.M., M.D.</i> . . . .	383
PHLORHIZIN DIABETES IN DOGS. <i>By F. H. Reilly, F. W. Nolan, and Graham Lusk</i> . . . . .	395
ON INTESTINAL ABSORPTION AND THE SALINE CATHARTICS. <i>By George B. Wallace and Arthur R. Cushny</i> . . . . .	411
THE MOVEMENTS OF THE FOOD IN THE ŒSOPHAGUS. <i>By W. B. Cannon and A. Moser</i> . . . . .	435
A CONTRIBUTION TO THE CHEMISTRY OF CYTOLOGICAL STAINING. <i>By Albert Mathews</i> . . . . .	445
NOTES ON CETRARIA ISLANDICA (ICELAND MOSS). <i>By Ernest W. Brown, Ph.D.</i> . . . .	455
VARIATIONS IN THE AMYLOLYTIC POWER AND CHEMICAL COMPOSITION OF HUMAN MIXED SALIVA. <i>By R. H. Chittenden and A. N. Richards, B.A.</i> . . . .	461
THE VENOMOTOR NERVES OF THE HIND LIMB. <i>By F. W. Bancroft</i> . . . .	477
AN ANALYSIS OF THE ACTION OF THE VAGUS NERVE ON THE HEART. <i>By L. J. J. Muskens</i> . . . . .	486
A NEW METHOD FOR THE STUDY OF THE ISOLATED MAMMALIAN HEART. <i>By W. T. Porter</i> . . . . .	511
PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY . . . .	iii-xv
INDEX . . . . .	519



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NO. I.

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THE INFLUENCE OF BORAX AND BORIC ACID UPON  
NUTRITION WITH SPECIAL REFERENCE TO  
PROTEID METABOLISM.

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IN view of the wide-spread use of borax and boric acid as food preservatives it is somewhat singular that our knowledge of the influence of these substances upon the nutritional processes of the body is so slight and uncertain. E. de Cyon,<sup>1</sup> M. Gruber,<sup>2</sup> and J. Forster<sup>3</sup> have indeed studied the action of these agents upon proteid metabolism, but with results which are utterly lacking in harmony. Thus Cyon's work with borax seemingly indicates that proteid metabolism is diminished under its influence, *i. e.*, that borax tends to protect the consumption of proteid matter in the tissues. Gruber's experiments, on the other hand, indicate with equal positiveness that borax has no proteid sparing power, but really leads to an increase in the rate of proteid metabolism. To add to the uncertainty, the experiments with boric acid carried out under Forster's supervision tend to show that this agent is wholly without influence upon proteid metabolism. Obviously, conclusions which are so much at variance cannot be accepted without careful consideration.

<sup>1</sup> CYON: Sur l'action physiologique du borax. Comptes rendus, 1878, tome 87, p. 845.

<sup>2</sup> GRUBER: Ueber den Einfluss des Borax auf die Eiweisszersetzung im Organismus. Zeitschr. f. Biol., 1880, Band 16, p. 198.

<sup>3</sup> FORSTER: Ueber die Verwendbarkeit der Borsäure zur Conservirung von Nahrungsmitteln. Nach Versuchen von Dr. G. H. Schlencker aus Surakarta. Archiv. f. Hygiene, 1884, Band 2, p. 75.

Cyon's experiments were conducted simultaneously on three full-grown dogs which were fed upon a diet almost exclusively proteid. His observations were practically limited to determining changes in body-weight during short periods, with an estimation of the nitrogen of the urine. He found that during the period when borax was included in the food, the animals gained noticeably in body-weight and that less nitrogen was contained in the excreta than in the ingesta. From these very crude observations the conclusion was drawn that borax, even to the extent of 12 grams per day, may be ingested with the food, especially when the latter is essentially proteid in nature, without provoking the slightest disturbance in general nutrition. Further, Cyon appeared to see in his results evidence that borax, if substituted for common salt in food, will facilitate the assimilation of the latter and bring about a great increase in the weight of the animal. Such deductions, however, were wholly unwarranted from the data at hand, for not only were the periods of observation exceedingly short, but, as pointed out by both Gruber<sup>1</sup> and C. Voit,<sup>2</sup> the animals at the beginning were much emaciated and received throughout the experiment such excessive quantities of meat that increase of body-weight would have inevitably followed without the presence of borax. Consequently, all that can be inferred legitimately from Cyon's experiments is that assimilation and general metabolism were not seriously affected by borax in the quantities given.

In Gruber's work more scientific methods were pursued, but it may well be questioned whether the conditions under which the experiments were conducted were adapted for bringing out clearly the full action of borax upon proteid metabolism. The two dogs employed were fed simply upon meat and water, and were presumably in a condition of nitrogenous equilibrium. In the first experiment, where the animal received daily 1500 grams of meat and 200 c.c. of water, the daily excretion of urea in the urine varied from 75.82 grams to 110.30 grams during the six days prior to the administration of borax. Then 20 grams of borax were introduced with the food, an amount so large that vomiting was at once produced, leading to a loss of about 5 grams of the borax and about 100 grams of the meat, with most of the water. On this day, however, 108.20 grams of urea were excreted in the urine, although the food consumed was 100 grams less than the usual quantity. On the two fol-

<sup>1</sup> GRUBER: *loc. cit.*

<sup>2</sup> VOIT: Hermann's *Handbuch der Physiologie*, Band 6, Theil I, p. 165.

lowing days, without borax and with the full complement of food, the excretion of urea amounted to 109.00 and 107.60 grams respectively. From these results Gruber concludes that the borax increased the excretion of urea 4-6 per cent. In the second experiment, with a dog of 34 kilos body-weight, fed on a daily ration of 1100 grams of meat and 200 c.c. of water, the daily excretion of urea varied from 70.86 grams to 80.60 grams for the four days of the normal period, while the administration of 10 grams of borax was accompanied by an excretion of 82.14 grams of urea, and, on the second day following, the introduction of 20 grams of borax was accompanied by an excretion of 85.25 grams of urea. Further, on this latter day the volume of urine rose to 1310 c.c., while the largest daily excretion prior to this day was 1040 c.c. Gruber, therefore, concludes that borax does not spare proteid as Cyon asserts, but, just as in the case of common salt, sodium sulphate, and other neutral salts, it causes an increase in the elimination of water from the body and induces therewith an increased proteid catabolism. It is not to be inferred from this statement that there is simply an increased washing out of urea from the tissues, for, as Voit<sup>1</sup> has pointed out, the amounts of urea excreted on the days following the ingestion of borax simply fall back to the neighborhood of the average for the normal period, and do not drop below that average. Gruber also concludes that borax has no unfavorable influence upon the assimilation of food, since the quantity of fæces, their content of solid matter and of nitrogen are within the limits of the normal elimination during periods when meat alone is fed. Further, no harmful influence, even after the ingestion of the largest dose — 20 grams — was to be observed, and the appetite of the animal was found to be undiminished on the days following that upon which borax was given. The objection we would make to accepting Gruber's conclusions in their entirety is that they are based solely upon the results following the administration of two large doses of borax, 10 and 20 grams, whereas, to our mind, longer periods with a dosage of borax continued for several days in succession would seemingly render the conditions much more favorable for an accurate judgment as to the character of the influence exerted by the substance on tissue changes. Further, since urea alone was determined in the urine, possible minor changes connected with the presence of the salt would naturally be overlooked. Lastly, we are inclined to the view that it is extremely

<sup>1</sup> VOIT: loc. cit. p. 165.

hazardous to draw such sweeping conclusions from one or two single experiments of this nature, especially where, as in the animal body, individual characteristics not infrequently give rise to exceptional results quite foreign to those ordinarily obtainable.

In Forster's work with boric acid, Dr. Schlencker experimented on himself, using a mixed diet and taking boric acid in daily doses of 1-3 grams. Each experiment consisted of three periods, of three days each, the boric acid being taken in the middle period. The conclusions arrived at were that proteid metabolism is not influenced, the excretion of urea in the boric-acid period being midway between that of the fore and after periods. It was noticed, however, that the quantity of ethereal sulphuric acid in the urine was considerably lessened in the boric-acid period and in the period following, thus implying an inhibitory influence upon the putrefactive processes of the intestine. Further, it was observed that the volume of the fæces, together with the contained nitrogen, was greatly increased under the influence of boric acid, from which it was inferred that this agent interferes with the assimilation of the food and perhaps, at the same time, gives rise to an increased secretion of mucus with a possible increase in the discharge of epithelial cells from the intestinal mucosa. This latter, however, is purely conjectural. Increased secretion of bile is also said to result from the action of boric acid. On the pulse and temperature no action was observed.

It is thus quite evident that the influence of borax and boric acid on nutrition, and especially their influence on proteid metabolism, is by no means wholly settled. The preceding statements clearly emphasize the uncertainty of our present information on the more essential features of the question before us, and we have therefore deemed it desirable to carry out, as thoroughly as possible, a series of experiments upon the action of both borax and boric acid on proteid metabolism and related phases of nutrition.

**Conduct of the Experiments.**—The experiments were conducted wholly upon full-grown dogs ranging in weight from 8 to 12 kilos. The animals were confined in suitable cages partially lined with galvanized iron and with the floor so arranged that both fluid and solid excreta could be collected in their entirety, while the upper portions of the cage were so constructed as to permit unrestricted circulation of air. In view of the length of the experiments—ranging from twenty-seven to fifty-six days each, with periods of eight to ten days duration—it seemed inadvisable as well as unnecessary to empty the

bladder each day with a catheter. Such diurnal variations as might possibly occur from incomplete emptying of the bladder at the end of the twenty-four hours would obviously be neutralized in periods of the above length, and consequently the urine was collected as naturally excreted, thus avoiding any possible disturbance of the normal condition of the bladder, etc. At the end of each twenty-four hours, the urine collected was combined, and its volume, specific gravity, etc., determined, after which the bottom of the cage was rinsed with a little distilled water and these washings added to the main fluid. The latter was then made up to some convenient volume in preparation for the daily analysis.

The fæces whenever passed were collected in a weighed dish, the mass thoroughly desiccated over a water-bath, and the dry weight ascertained. The dried material was then pulverized and the nitrogen-content as well as the ether-soluble matter determined in sample portions. The nitrogen determinations were always made in duplicate by the Kjeldahl method and rarely varied more than 0.05 per cent. Whenever, as sometimes occurred, hair accumulated in the cage it was likewise collected and the nitrogen determined. The ether-soluble matter was determined by extraction of the dried fæces in a Soxhlet-apparatus.

The animals were fed during the experiments on a mixed diet composed of fresh lean beef, cracker dust, lard, and water. The meat was prepared as follows: fresh lean beef, freed as far as possible from all adherent fat and connective tissue, was run through a hashing machine, after which it was enclosed in a bag of thin cloth, placed under a heavy press, and kept there under increasing pressure for several hours, the bloody fluid which drained off being thrown away. By this method there results a mass of tissue free from surplus moisture, and which, when enclosed in a bottle, will keep perfectly fresh on ice for seven to ten days without separation of fluid. Several advantages accrue from this method. Thus, we have a perfectly homogeneous mixture which can be drawn from for at least a week with surety that its nitrogen-content is constant. There is therefore no necessity for a daily determination of nitrogen in this portion of the diet, for each sample can be analyzed when prepared and the data accepted as long as the meat keeps fresh. Further, meat prepared in this manner at different times, if subjected to essentially the same pressure, varies only slightly in its content of nitrogen. We have invariably analyzed each lot when prepared to avoid any pos-

sibility of error, but, as the following results show, the differences in composition are very slight and necessitate very little alteration in the proportion of meat when changing from one lot to another. The following results are a few of the many obtained:

	Weight of Meat.	Absolute Content of Nitrogen.	Percentage of Nitrogen.
1.	0.8703 gram	0.03041 gram	3.49
	0.7710 "	.02682 "	3.48
	0.7631 "	.02628 "	3.44
2.	0.7673 gram	0.02716 gram	3.54
	0.9228 "	.03238 "	3.51
	1.0591 "	.03723 "	3.52
3.	0.8478 gram	0.03015 gram	3.56
	1.0014 "	.03591 "	3.59
	0.8876 "	.03152 "	3.55
4.	1.0082 gram	0.03642 gram	3.61
	1.0445 "	.03783 "	3.62
	1.0803 "	.03961 "	3.67
5.	1.1977 gram	0.04265 gram	3.56
	0.8142 "	.02902 "	3.56
	0.9793 "	.03463 "	3.54

The carbohydrate element in the diet, as already stated, was supplied by commercial cracker dust. This was purchased in large quantity and preserved in well stoppered bottles. It contained on an average 1.46 per cent of nitrogen. The lard employed was entirely free from any recognizable amount of nitrogen.

The daily diet was divided into two equal portions, one-half being fed at 8 A.M. and the other half at 6 P.M. When borax or boric acid was given, the daily dose was likewise divided and given either with the food or directly after. The body-weight of the animal was taken each morning just before feeding. Each day's urine included the fluid passed from 8 A.M. to 8 A.M. of the next day.

**Methods of Analysis.**—Nitrogen was determined wholly by the Kjeldahl method, viz., in the daily analyses of the urine, fæces, and food material. All analyses were made in duplicate, and the figures given are based upon the average of closely agreeing results. In analysis of the urine 5 c.c. were used for each determination, oxida-

tion being carried out in a long-necked Kjeldahl flask with 10 c.c. of sulphuric acid and a crystal of cupric sulphate, thus doing away with the necessity of adding sodium sulphide in the distillation. The ammonia formed was distilled into quarter-normal hydrochloric acid, the latter being titrated with quarter-normal ammonia, using congo red as an indicator.

Sulphur and phosphorus were determined in the customary manner by evaporating a given volume of the urine—25 c.c. for each determination—in a roomy silver crucible with 10 grams of pure sodium hydroxide (made from the metal) and 2 grams of potassium nitrate, igniting the residue until oxidation was complete and treating the fused mass with water. For sulphur, the mixture was acidified with hydrochloric acid, evaporated to dryness, the residue moistened with a few drops of hydrochloric acid and dissolved in hot water. The filtered solution was then precipitated in the usual manner with barium chloride, the resultant barium sulphate filtered, ignited, and weighed, thus giving data for calculation of the total sulphur. For phosphorus, the aqueous extract of the oxidized urine was acidified with nitric acid, evaporated to dryness, the residue moistened with nitric acid and dissolved in warm water. From this solution the phosphoric acid was precipitated in the usual manner with molybdic solution and eventually transformed into ammonio-magnesium phosphate. From the weight of magnesium pyrophosphate obtained, the total phosphorus of the urine was calculated.

Uric acid was determined by the well-known Salkowski-Ludwig silver method, using 100–200 c.c. of urine.

Phosphoric acid was determined by Mercier's<sup>1</sup> modification of Neubauer's method, *i. e.* by titration of 50 c.c. of urine with a standard solution of uranium nitrate and tincture of cochineal as an indicator.

Total sulphuric acid was estimated by diluting 25 c.c. of urine with 3–4 volumes of water, adding 5 c.c. of dilute hydrochloric acid, heating to boiling, and precipitating hot with barium chloride. The barium sulphate so obtained, after standing twenty-four hours in a warm place, was washed with hot water until free from chlorides and lastly with hot alcohol, ignited, and weighed.

Combined sulphuric acid was determined by Baumann's method, using 100 c.c. of urine.<sup>2</sup>

<sup>1</sup> See Neubauer und Vogel's *Analyse des Harns*, neunte Auflage, p. 450.

<sup>2</sup> *Ibid.*, p. 447.

Chlorine was determined in 10 c.c. of urine by Neubauer and Salkowski's modification of Mohr's method.<sup>1</sup> Other methods occasionally made use of are referred to in their appropriate place.

**First Experiment. With Borax.** — The animal made use of in this experiment was a short-haired mongrel bitch weighing about 12 kilos. She was brought into a condition approximating to nitrogenous equilibrium only after a preliminary period of nearly three weeks, during which time superfluous fat was lost and she became wholly accustomed to her surroundings. The daily food, at the time the experiment actually commenced, consisted of 250 grams of the prepared meat, 70 grams of cracker dust, 40 grams of lard, and 500 c.c. of water. It contained 9.814 grams of nitrogen. This diet, with the above content of nitrogen, was adhered to throughout the entire experiment of twenty-seven days, the only variation being the slight changes in the amount of nitrogen, to be seen in the tables, incidental to the use of different lots of meat and in the employment of gelatin capsules during the borax period. These gelatin capsules, in which the borax was administered, contained 14.95 per cent of nitrogen, the four capsules used each day during the borax period containing 0.12 gram of nitrogen. This amount was naturally included in the nitrogen of the food.

The experiment extended through twenty-seven days and was divided into three periods of nine days each: a fore or normal period during which no borax was given, a borax period during which 45 grams of borax (5 grams a day) were administered, and an after period when normal conditions again prevailed. During the borax period of nine days the quantity of borax given per day amounted to nearly 0.6 per cent of the total food and drink ingested, while of the solid food it formed 1.3 per cent. This dosage of borax, considering the size of the animal, was fairly large, and with this particular dog considerable difficulty was experienced in inducing the animal to take it. At first the borax was simply mixed with the food, but its presence was quickly detected and the food refused, although it was eventually coaxed down, but with some difficulty. After this first day the borax was given in capsules, as already stated, and no further difficulty of this sort was experienced. Three times during the borax period, however, the animal was nauseated and vomited a portion of the food, thus showing that this quantity of borax was sufficient to disturb the physiological equilibrium of the animal.

<sup>1</sup> See Neubauer und Vogel's *Analyse des Harns*, neunte Auflage, p. 437.



The vomited matter was eventually eaten, however, later in the day, so that this occurrence did not disturb the validity of the experiment. It will be remembered that in Gruber's experiment with a much larger dog (39 kilos) 20 grams of borax likewise caused vomiting. In his experiment, however, the entire dose of borax was taken at one time, while in our case, 2.5 grams were given in the morning and a like quantity at night. Hence, taking into account the weight of the dog, it might perhaps be argued that 0.25 gram of borax to 1 kilo of body-weight will produce vomiting. This, however, is very questionable, for in the above experiment the dog did not vomit until the afternoon of December 5, when she had already taken 12.5 grams of borax. In other words, the animal was without doubt suffering in part from the cumulative action of the salt. Thus, there was a slight attack of vomiting again on the fifth day (December 7) and a final attack on the eighth day (December 10). During the after period of nine days the animal was perfectly normal, and at the close of the period, to again test the action of the borax, 5 grams were given at one time shortly after the morning meal. Forty-five minutes afterwards the animal vomited, and this occurred three times during the forenoon. We are inclined to lay particular emphasis upon this action of the borax because it tends to show that in this first experiment the dosage of borax through the nine days' period was as large as it well could be for this particular animal without vitiating the experiment, and that the conditions were therefore well adapted for bringing out distinctly any possible influence the borax might have upon the metabolic phenomena of the body. Further, we would call attention to the obvious advantage — in spite of the greater labor involved — of continuing experiments of this character over comparatively long periods of time. To be sure, in some cases where the substance being tested has a marked physiological action, a single dose may show at once the character of the influence exerted, but too often erroneous conclusions are arrived at through negligence of this precaution. Where, however, the substance under examination is given for five to ten days consecutively, with careful examination of the excreta, the chances of detecting minor influences are greatly increased, and at the same time the danger of being led astray by a single exceptional result — or by other possible errors — is greatly diminished.

The following tables contain the analytical results obtained throughout the experiment.

## FIRST EXPERIMENT. — FORE PERIOD.

DATE.		BODY.		FOOD.		URINE.							FÆCES.									
1896.	Weight.	Nitrogen.		Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Phos- phorus.	Sulphur.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Dry Weight.	Nitrogen.								
	kilos	grams	c.c.												litmus							
Nov.											grams											
24	10.9	9.814	505	1018	Acid.	7.945	0.035	0.468	0.491	0.962	0.058	....	....	....								
25	10.9	9.814	716	1018	"	11.361	.049	.646	.720	1.388	.075	....	....	....								
26	10.9	9.814	773	1017	"	11.367	.061	.688	.671	1.343	.077	....	....	....								
27	11.0	9.814	786	1016	"	12.476	.049	.763	.737	1.521	.084	....	....	....								
28	11.0	9.814	650	1017	"	10.069	.047	.585	.586	1.214	.064	....	....	....								
29	11.0	9.814	415	1017	"	6.102	.040	.325	.381	0.765	.032	....	....	....								
30	10.8	9.814	770	1019	"	12.302	.066	.760	.758	1.554	.084	....	....	....								
Dec. 1	10.9	9.814	575	1017	"	8.995	.040	.505	.570	1.148	.062	....	....	....								
2	11.0	9.814	439	1018	"	6.568	.038	.410	.405	0.804	.055	38.15	2.122	2.122								
					Nitrogen of Urine = 87.185 Nitrogen of Fæces = 2.122																	
Totals		88.326		5629	89.307										0.428	5.150	5.319	10.699	0.591	38.15	2.122	
Daily Averages		9.814		625	9.923										0.048	0.572	0.591	1.189	0.066			0.236

FIRST EXPERIMENT.—BORAX PERIOD.

DATE.	BODY.		FOOD.		BORAX.	URINE.								FÆCES.			
	Weight.	kilos	Nitrogen.	grams		Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Phos-phorus.	Sulphur.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Dry Weight.	Nitro-gen.	
1896.					c.c.	litmus						grams					
Dec.																	
3	10.9	9.903	5	796	1021	Acid.	13.344	0.054	0.821	.789	1.631	0.097	....	....	....	....	
4	11.1	9.933	5	368	1022	Alkaline.	5.909	.032	.321	.371	0.705	.039	....	....	....	....	
5	11.2	9.933	5	485	1025	"	9.183	.039	.535	.527	1.103	.057	....	....	....	....	
6	11.1	9.933	5	520	1027	"	10.043	.042	.568	.592	1.197	.060	....	....	....	....	
7	11.1	10.016	5	686	1024	"	12.823	.050	.818	.754	1.526	.068	35.91	2.292	2.292	2.292	
8	11.2	10.100	5	422	1024	"	7.412	.051	.444	.426	0.825	.042	....	....	....	....	
9	11.2	10.100	5	604	1023	"	10.742	.049	.615	.596	1.228	.069	....	....	....	....	
10	11.3	10.100	5	498	1026	"	9.846	.031	.521	.600	1.174	.060	....	....	....	....	
11	11.3	10.100	5	602	1020	"	8.825	.063	.456	.554	1.040	.063	24.68	1.627	1.627	1.627	
				Nitrogen of Urine = 88.127													
				Nitrogen of Fæces = 3.919													
Totals				45	4981	92.046			0.411	5.099	5.209	10.429	0.555	60.59	3.919	3.919	
Daily Averages				5	553	10.227			0.046	0.567	0.579	1.159	0.062		0.435	0.435	

## FIRST EXPERIMENT. — AFTER PERIOD.

DATE	BODY.		FOOD.		URINE.							FÆCES.	
	Weight	Nitrogen.	Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Phosphorus.	Sulphur.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Dry Weight.	Nitrogen.
1896.	kilos	grams			litmus								
Dec.			c c.										
12	11.5	9.981	488	1019	Acid.	8.727	0.042	0.441	0.596	1.024	0.055	....	....
13	11.3	9.981	670	1018	"	10.632	.053	.589	.716	1.247	.073	. .	....
14	11.4	9.981	691	1017	"	10.047	.039	.621	.742	1.265	.083	....	....
15	11.5	9.981	551	1016	"	7.804	.032	.482	.601	0.978	.049	....	....
16	11.4	9.981	681	1018	"	10.549	.051	.694	.736	1.345	.073	33.25	1.995
17	11.5	9.981	595	1019	"	10.121	.036	.662	.662	1.213	.062	....	....
18	11.5	9.981	572	1018	"	9.232	.036	.587	.613	1.119	.069	....	....
19	11.5	9.981	630	1017	"	9.587	.056	.574	.610	1.083	.068	....	....
20	11.5	10.036	549	1019	"	9.678	.044	.574	.616	1.180	.069	25.45	1.629
					Nitrogen of Urine = 86.377 Nitrogen of Fæces = 3.624								
Totals	. . .	89.884	5427			90.001	0.389	5.224	5.892	10.454	0.601	58.70	3.624
Daily Averages		9.987	603			10.000	0.043	0.580	0.655	1.162	0.067		0.403

FIRST EXPERIMENT. — GENERAL SUMMARY.

PERIODS.	TOTAL NITROGEN.			URINE.							FÆCES.	
	Ingested.	Excreted.	Balance.	Vol.	Nitrogen.	Uric Acid.	Phos- phorus.	Sulphur.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Dry Weight.	Nitrogen.
grams												
c.c.												
Fore . .	88.326	89.307	— 0.981	5629	87.185	0.428	5.150	5.319	10.699	0.591	38.15	2.122
Borax . .	90.118	92.046	— 1.928	4981	88.127	.411	5.099	5.209	10.429	.555	60.59	3.919
After . .	89.884	90.001	— 0.117	5427	86.377	.389	5.224	5.892	10.454	.601	58.70	3.624

Referring now to the tables containing the results of the first experiment, it is to be noted that in the fore period of nine days the total nitrogen ingested amounted to 88.326 grams, while in the urine excreted during this period there were contained 87.185 grams of nitrogen, and in the faeces 2.122 grams, making a total of 89.307 grams of nitrogen; hence the nitrogen balance for the period of nine days is —0.981 gram. The body-weight remained practically constant. The slight excess of nitrogen excreted over the amount ingested in this period is due possibly to lack of complete involution of the mammary glands<sup>1</sup>; the deficiency, however, is too slight considering the length of the period, to need much consideration. For comparison, the results of the three periods, showing the relative excretion of nitrogen, may be arranged in tabular form:

	<i>Fore Period.</i>	<i>Borax Period.</i>	<i>After Period.</i>
Nitrogen of Food . . . . .	88.326	90.118	89.88
Nitrogen of Urine . . . . .	87.185	88.127	86.377
Nitrogen of Faeces . . . . .	2.122	3.919	3.624
	89.307	92.046	90.00
Nitrogen Balance . . . . .	—0.981	—1.928	—0.11
Ratio of Urine Nitrogen to Food Nitrogen . . . . .	98.6 per cent.	97.7 per cent.	96.0 per cent.

It is thus evident that in this experiment, in spite of the large doses of borax and the length of the period, proteid metabolism is not modified in any noticeable degree. The amount of nitrogen eliminated through the urine in proportion to the nitrogen of the food, during the borax period, differs from that of the fore period only to a slight extent, and this difference is due apparently to a diminished assimilation of the proteid food. The change in the nitrogen balance of the borax period is plainly caused by a slight increase in the amount of faecal nitrogen, and not to increased metabolism, thus indicating that the borax has a tendency to diminish somewhat the absorption of proteid food, or possibly leads to an increased secretion of mucus. When, however, the nitrogen of the faeces of the borax period is compared with both that of the fore and after periods the increase is seen to be so slight that it is perhaps unwise to attach much importance to it. Certainly the borax though given in doses sufficiently large to keep the animal on the verge of nausea, did not in this experiment interfere greatly with the

<sup>1</sup> MARCUSE: Ueber den Nährwerth des Caseins, Pflüger's Archiv. f. d. ges. Physiol., 1896, Band 64, p. 223.

digestion of any of the food-stuffs, since the fæces of the borax period are not much greater in amount than those of the after period, though somewhat larger than those of the fore period.

The weight of the animal during the twenty-seven days' period showed a tendency to rise somewhat, *i. e.*, from 10.9 kilos to 11.5 kilos. This, however, is not to be attributed to a laying on of fat nor to a retention of nitrogenous matter by the body, but is the result simply of a diminished excretion of water due to the presence of the borax. The results in this connection are in direct opposition to those obtained by Gruber with single doses of borax. There is here no suggestion whatever of an increased excretion of water, but on the contrary, a very marked decrease. Thus, by reference to the accompanying tables, it will be observed that during the fore period the total volume of urine excreted amounted to 5629 c.c. and the body-weight remained practically constant, *i. e.*, 10.9-11.0 kilos. During the borax period, however, the volume of urine excreted fell to 4981 c.c. and the body-weight gradually rose to 11.3 kilos, while in the after period the volume of urine rose to 5427 c.c. with a constant body-weight of 11.5 kilos. It is thus quite clear that borax may decidedly check the output of water through the kidneys, and lead, as in this case, to its retention within the body.

Very noticeable also, in this experiment, is the sudden change in the specific gravity of the urine, as also in the reaction of the fluid, when borax is given. Thus, in the fore period the specific gravity of the urine stood at 1017-1018, but at the opening of the borax period it rose at once to 1022-1027, dropping back, however, as the borax was discontinued. Similarly, the reaction of the normal urine was acid to litmus, but on exhibition of borax, the reaction quickly changed to alkaline. The marked rise in the specific gravity of the urine during the borax period is not due solely to diminished elimination of water nor to increase in the proportion of metabolic products, but mainly to the borax itself, which is rapidly eliminated through the urine. We have not made any special trial to ascertain how soon the borax appears in the urine after its administration, but we have observed that the urine collected on the first day of the borax period gives, after acidulation with hydrochloric acid, a strong reaction with turmeric paper for boric acid. Further, that the elimination of borax through the urine is very rapid is manifest from the fact that, at the end of the borax period, the animal having received 45 grams of the salt, no trace of a reaction could be obtained with

turmeric paper on the *second* day of the after period. In other words, elimination of the borax was practically complete twenty-four to thirty-six hours after the last dose had been taken. These observations accord with Johnson's statements<sup>1</sup> that borax and boric acid begin to be eliminated through the urine a short time after their administration.

While it is clear from a study of the nitrogen excretion that proteid metabolism, under the conditions of this experiment, is not materially affected by borax, the other analytical results must not be overlooked. Thus, in the borax period the excretion of phosphorus, sulphur, total sulphuric acid, and combined sulphuric acid is slightly below that of the fore and after periods. The differences, however, are so small that it is perhaps unwise to draw any positive conclusions from them, other than to admit their negative character. It can certainly be asserted with perfect safety that the borax has failed to exert any marked influence upon the excretion of either sulphur or phosphorus. In this connection it will be remembered that Forster<sup>2</sup> found, on feeding boric acid to man, a marked increase in the output of phosphoric acid. Borax, however, certainly fails to produce any such result, its presence in the body (of the dog) tending on the other hand to reduce the output of phosphorus. Further, it is evident that the slight diminution in the excretion of combined sulphuric acid is not sufficient to indicate any inhibitory influence upon intestinal putrefaction. Lastly, the figures obtained in connection with uric acid are such as to indicate a purely negative action.

**Second Experiment. With Boric Acid.**—The animal experimented on was a short-haired mongrel bitch weighing 8 kilos. Nitrogenous equilibrium was quickly established on a daily diet composed of 160 grams of the prepared meat, 40 grams of cracker dust, 30 grams of lard, and 400 c.c. of water. This diet contained 6.144 grams of nitrogen and was practically adhered to throughout the experiment. The latter was of thirty days' duration, *i. e.*, three periods of ten days each. During the middle, or boric acid period, 1–2 grams of boric acid were given daily mixed with the food, the animal taking it without

<sup>1</sup> JOHNSON: Ueber die Ausscheidung von Borsäure und Borax aus dem menschlichen Organismus. Jahresbericht f. Thierchemie, 1885, p. 235. See also, VIGIER: Note préliminaire sur l'action physiologique du borate de soude. Comptes rendus soc. de Biol. Paris, 1883, p. 44.

<sup>2</sup> FORSTER: Archiv. f. Hygiene, 1884, Band 2, p. 75.



the slightest reluctance and without any apparent effect upon the appetite. No sign of nausea or vomiting was seen. With 2 grams of boric acid per day the mixture of food and drink contained 0.31 per cent, while the dry food contained 0.86 per cent of boric acid. The total amount of boric acid given during the ten days was 14.5 grams.

During the fore period of ten days the animal received a total of 61.440 grams of nitrogen. The nitrogen excreted through the urine for this period amounted to 58.119 grams, while the fæces contained 3.203 grams, thus making a total of 61.322 grams of nitrogen excreted, with a nitrogen balance of +0.118 gram. Plainly, the animal was in a condition of nitrogenous equilibrium.

The relative excretion of nitrogen for the three periods may be seen in the following table:

	<i>Fore Period.</i>	<i>Boric Acid Period.</i>	<i>After Period.</i>
Nitrogen of Food . . . . .	61.440	62.032	61.943
Nitrogen of Urine . . . . .	58.119 } 61.322	59.600 { 63.538	58.979 } 62.923
Nitrogen of Fæces . . . . .	3.203 }	3.938 }	3.944 }
Nitrogen Balance . . . . .	+ 0.118	— 1.506	— 0.980
Ratio of Urine Nitrogen to Food Nitrogen . . . . .	94.5 per cent.	96.7 per cent.	95.2 per cent.

From these figures it would appear that there is a slight tendency toward stimulation of proteid metabolism. When it is remembered, however, that the nitrogen balance for the boric acid period, —1.506, is the result of ten days' consecutive feeding with boric acid, it is manifest that the stimulating action is very slight, and our results may perhaps be considered as practically in accord with those reported by Forster, who found that in man on a mixed diet, boric acid in moderate doses (1–3 grams) was without influence on proteid decomposition as measured by the excretion of urea. Upon the assimilation of the proteid food there is no evidence of any action, *i. e.*, the nitrogen content of the fæces during the boric acid period is essentially the same as that of the fore and after periods. Further, the total weight of fæces for each of the three periods is so nearly the same, it is quite evident that assimilation has not been materially interfered with. In this respect our results fail to agree with those reported by Forster, who found that small doses of boric acid (1 gram in two days) given to a man on a mixed diet, and on a milk and egg diet, increased the excretion of fæces; this increase being due,

according to Forster, not to any decrease in the assimilation of fat nor to increase in the volume of the secretions, but to a decreased assimilation of the proteid food under the influence of the boric acid. This difference in our results may of course depend upon the difference in the character of the animal species. In our experiment, the weight of the animal remained perfectly constant throughout the entire period of thirty days.

The accompanying tables contain the various data obtained.

Unlike borax, boric acid fails to produce any change in the volume of the urine. Thus, in the fore period of ten days the total volume excreted amounted to 4647 c.c., while in the boric acid period of the same length the total volume was 4665 c.c., and in the after period 4644 c.c. Further, there is no marked difference, to be measured by litmus paper, in the reaction of the fluid, although, as the tables show, alkaline reaction is more common in the normal periods than in the boric acid period. In the latter period, however, the specific gravity of the urine, as might be expected, shows a higher average than in the two normal periods. This is due, as in the case of borax, to the rapid elimination of the boric acid through the urine. The latter shows the presence of the acid by the turmeric test on the first day of the boric acid period, while on the second day of the after period all trace of a reaction disappears, thus showing that the acid is rapidly eliminated from the body and is practically completely removed twenty-four to thirty-six hours after the last dose.

Upon the elimination of uric acid, boric acid appears to have a slight inhibitory effect, at least under the conditions of this experiment, but upon the excretion of total and combined sulphuric acid, chlorine and phosphoric acid, no tangible effect is produced. Certainly, the results in connection with combined sulphuric acid do not indicate any retarding effect upon the putrefactive processes of the intestine. In this connection it will be remembered that in Forster's experiments on man doses of boric acid, corresponding to those used by us, apparently gave rise to a marked retardation in the amount of ethereal sulphate excreted. As a result, Forster arrived at the conclusion that boric acid materially reduces intestinal putrefaction. Our results, however, show no action of this kind in the dog, and we are inclined to the view that both borax and boric acid are too rapidly eliminated from the system to be very effective in the intestine. As already stated, the elimination of borax and boric acid through the

SECOND EXPERIMENT.—FORE PERIOD.

DATE.	BODY.		FOOD.		URINE.								FÆCES.				
	1897. Feb.	Weight.	Nitrogen.	grams	Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Chlorine.	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitro- gen.		
		kilos														c.c.	litmus
24	7.9		6.144		500	1015	Acid.	6.642	0.037	0.682	0.023	0.354	0.950	....	....		
25	7.9		6.144		486	1012	"	5.051	.055	.533	.021	.404	0.726	....	....		
26	7.9		6.144		460	1014	"	5.741	.048	.638	.029	.340	0.789	6.96	0.450		
27	8.0		6.144		410	1015	"	4.956	.049	.560	.024	.379	0.665	....	....		
28	7.9		6.144		581	1014	"	7.605	.096	.830	.036	.573	1.053	....	....		
Mar. 1	8.0		6.144		325	1014	"	4.067	.033	.477	.020	.210	0.506	....	....		
2	7.9		6.144		525	1016	Alkaline.	7.613	.052	.807	.040	.404	1.008	11.90	0.780		
3	7.9		6.144		440	1014	"	5.425	.057	.581	.022	.407	0.722	10.50	0.657		
4	8.0		6.144		370	1014	"	4.119	.026	.464	.016	.291	0.540	....	....		
5	7.9		6.144		550	1015	Acid.	6.900	.051	.749	.026	.462	1.004	17.30	1.316		
					Nitrogen of Urine = 58.119 Nitrogen of Fæces = 3.203												
Totals		. . .	61.440		4647			61.322	0.504	6.321	0.257	3.824	7.963	46.66	3.203		
Daily Averages			6.144		465			6.132	0.050	0.632	0.026	0.382	0.796		0.320		

## SECOND EXPERIMENT. — BORIC ACID PERIOD.

DATE.		BODY.		FOOD.		BORIC ACID.		URINE.							FÆCES.	
1897.	Mar.	Weight.	kilos	Nitrogen.	grams	Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Chlo- rine.	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitro- gen.
		c.c.		litmus		grams										
6		7.9		6.144		1	470	1016	Acid.	5.915	0.040	0.678	0.025	0.347	0.911	....
7		7.9		6.144		1	505	1016	"	6.390	.031	.712	.032	.451	0.946	....
8		8.0		6.183		1	380	1014	"	4.479	.028	.511	.020	.259	0.550	0.710
9		7.9		6.223		1	525	1017	"	7.280	.041	.767	.034	.356	1.073	....
10		8.0		6.223		1.5	400	1016	Alkaline.	+166	.026	.481	.017	.333	0.586	0.660
11		7.9		6.223		1.5	530	1017	Acid.	7.460	.062	.803	.034	.555	1.017	....
12		7.9		6.223		1.5	460	1017	"	6.000	.040	.664	.031	.538	0.849	1.317
13		7.9		6.223		2	470	1017	"	6.035	.041	.682	.031	.565	0.841	....
14		7.9		6.223		2	480	1017	"	6.032	.035	.648	.027	.488	0.828	0.882
15		7.9		6.223		2	445	1017	"	5.843	.042	.683	.027	.425	0.899	0.369
								Nitrogen of Urine = 59.600 Nitrogen of Fæces = 3.938								
Totals				62.032		14.5	4665		63.538	0.386	6.629	0.278	4.317	8.500	53.30	3.938
Daily Averages				6.203		1.45	467		6.354	0.039	0.663	0.028	0.432	0.850		0.394



## SECOND EXPERIMENT. — GENERAL SUMMARY.

PERIODS.	TOTAL NITROGEN.			URINE.							FÆCES.	
	Ingested.	Excreted.	Balance.	Vol.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Chlorine	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitrogen.
grams			c c.	grams								
Fore . . .	61.440	61.322	+ 0.118	4647	58.119	0.504	6.321	0.257	3.824	7.963	46.66	3.203
Boric Acid .	62.032	63.538	— 1.506	4665	59.600	.386	6.629	.278	4.317	8.500	53.30	3.938
After . . .	61.943	62.923	— 0.980	4644	58.979	.508	6.811	.316	4.232	8.449	55.60	3.944

urine commences almost immediately after their ingestion, and it is very questionable, therefore, whether, with moderate doses of these substances, enough would remain unabsorbed at the lower end of the small intestine to exert much influence upon the growth and development of micro-organisms. Certainly, the fæces do not ordinarily contain any appreciable amount of borax or boric acid after these substances have been administered in moderate quantities, although obviously the length of time the fæces are forming will have some influence upon their content of soluble matter. In only one instance, to be detailed later, where a very large dose of borax was given, could any decided reaction for boric acid be obtained in the fæces. Johnson<sup>1</sup> states that in the case of the human organism borax and boric acid show great irregularity in their appearance in the fæces, and that he was able to detect them in the latter only in six cases out of fourteen, although daily doses of 0.9–3.0 grams of boric acid were given.

Lastly, it is to be noted that in our experiment with boric acid there is no such increase in the excretion of phosphoric acid through the urine as was observed by Forster; our results, indeed, fail to show any distinct influence exerted by boric acid upon the metabolism of phosphorized matter.

**Third Experiment. With Borax and Boric Acid.**—This experiment was divided into seven periods of eight days each, thus making a total of fifty-six consecutive days during which the variations in the composition of the urine and fæces were followed as before, under the influence of both borax and boric acid. The object in extending the experiment through this lengthy period was to ascertain whether prolonged treatment with borax and boric acid might not eventually result in such a disturbance of physiological equilibrium that more positive data would be obtained. With this end in view, a mongrel bitch of ten kilos body-weight was brought into nitrogenous equilibrium, after which the urine and fæces were analyzed for eight consecutive days, *i. e.*, the fore period. Borax was then given with the food for eight days, making the first borax period. This was followed by another period of eight days during which neither borax nor boric acid were administered, after which came a third period of eight days when boric acid was fed. This, in turn, was succeeded by a normal period of equal length, followed by eight days of borax treatment—

<sup>1</sup> JOHNSON: Ueber die Ausscheidung von Borsäure und Borax aus dem menschlichen Organismus. Jahresbericht f. Thierchemie, 1885, p. 235.

the second borax period—concluding with a final after period of eight days, *i. e.*, a total of fifty-six days. By thus keeping the same animal under continuous observation for this length of time it might reasonably be expected that any cumulative action—assuming it to exist—would be clearly manifest. Further, considerably larger daily doses of borax and boric acid were administered than in the preceding experiments.

The daily diet made use of throughout the entire experiment consisted of 160 grams of the prepared meat, 40 grams of cracker dust, 30 grams of lard, and 430 c.c. of water. Its exact content of nitrogen is shown in the table of the fore period. The total amount of nitrogen ingested during the fore period was 52.163 grams. The amount excreted during the same period was 51.734 grams, thus showing a nitrogen balance for the eight normal days of +0.429 grams. The dog used in this experiment, although short-haired, lost considerable hair daily. This was therefore collected and at the end of each period its content of nitrogen was determined and the amount added to the nitrogen of the urine and faeces, as seen in the accompanying tables. It is interesting to note in this connection that the loss of hair in periods of eight days' duration may be considerable; so large, indeed, that an appreciable loss of nitrogen may result. Thus, in the seven periods of this experiment the total amount of hair shed was 61.98 grams, *i. e.*, 8–10 grams for each period, the total nitrogen thrown off in this manner amounting to 7.856 grams. These figures show that the hair shed contained 12.6 per cent of nitrogen. Obviously, in careful experiments, this source of loss cannot be overlooked.

In the first borax period of eight days the daily dose of borax ranged from 2 to 5 grams, the total amount administered being 32.5 grams. In the following boric acid period the daily dose ranged from 1 to 3 grams, a total of 17 grams of boric acid being given. On commencing the second borax period the daily dose of borax was placed at 10 grams. This was continued for two days, but on the third day after taking the morning dose of 5 grams the animal's appetite began to fail so that it became necessary to coax her considerably in order to have the day's ration consumed. On this day therefore, only 5 grams were given, but on the following day the appetite was nearly normal and 6 grams of borax were given. The dose was then raised to 10 and 8 grams daily, as shown in the tables, a total of 64 grams of borax being given in this period of eight days.



THIRD EXPERIMENT.—FORE PERIOD.

DATE.	BODY.		FOOD.		URINE.							FÆCES.	
	Weight.	Nitrogen.	Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Total P <sub>2</sub> O <sub>6</sub> .	Dry Weight.	Nitrogen.	
													kilos
1897.													
April.													
21	10.0	6.593	490	1015	Acid.	6.160	0.040	0.525	0.042	0.981	....	....	
22	10.0	6.770	470	1013	"	5.050	.032	.437	.036	0.697	....	....	
23	10.0	6.770	540	1016	"	7.139	.042	.641	.071	1.117	....	....	
24	10.1	6.406	440	1014	"	5.231	.028	.489	.059	0.779	....	....	
25	10.0	6.406	640	1015	"	7.685	.060	.746	.093	1.329	14.33	0.846	
26	10.0	6.406	465	1012	"	4.643	.031	.420	.039	0.638	....	....	
27	10.0	6.406	525	1014	"	5.641	.047	.544	.061	0.862	....	....	
28	9.9	6.406	626	1015	"	7.544	.035	.713	.094	1.335	10.36	0.571	
			Nitrogen of Urine = 49.093										
			Nitrogen of Fæces = 1.417										
			Nitrogen of Hair = 1.224										
Totals			4196			51.734	0.315	4.515	0.495	7.738	24.69	1.417	
Daily Averages			525			6.467	0.039	0.564	0.062	0.967		0.177	

## THIRD EXPERIMENT. — FIRST BORAX PERIOD.

DATE.		BODY.	FOOD.		URINE.						FÆCES.			
1897.		Weight.	Nitrogen.		Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitrogen.
April.		kilos	grams											
29	10.0		6.406	2	400	1015	Alkaline.	4.025	0.039	0.401	0.049	0.597	2.96	0.163
30	10.1		6.406	3	400	1022	"	6.738	.043	.677	.091	1.142	....	....
May 1	10.0		6.406	4	591	1018	"	6.542	.042	.704	.089	1.107	....	....
2	10.0		6.406	4	470	1021	"	7.028	.042	.797	.126	1.089	....	....
3	9.9		6.406	4.5	520	1017	"	5.916	.031	.565	.072	0.781	20.10	0.990
4	10.1		6.285	5	380	1017	"	4.041	.024	.372	.040	0.409	....	....
5	10.1		6.285	5	460	1022	"	6.531	.040	.597	.082	0.977	....	....
6	10.0		6.285	5	540	1022	"	7.503	.032	.792	.113	1.272	20.69	1.023
			Nitrogen of Urine = 48.324											
			Nitrogen of Fæces = 2.176											
			Nitrogen of Hair = 1.186											
Totals . . .			50.885	32.5	3761			51.686	0.293	4.905	0.662	7.374	43.75	2.176
Daily Averages			6.361	4.06	470			6.461	0.037	0.613	0.083	0.922		0.272

THIRD EXPERIMENT. — FIRST AFTER PERIOD.

DATE.	BODY.		FOOD.		URINE.							FÆCES.		
	Weight.	kilos	Nitrogen.	grams	Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitrogen.
1897.														
May.														
7	10.1		6.285		410	1015	Acid.	5.687	0.036	0.575	0.054	0.810	....	....
8	10.2		6.285		430	1012	"	4.330	.034	.449	.040	0.458	....	....
9	10.1		6.285		590	1016	"	7.671	.044	.623	.117	1.187	....	....
10	10.2		6.428		390	1014	"	4.717	.014	.563	.065	0.599	....	....
11	10.0		6.428		597	1015	"	7.425	.036	.872	.106	1.423	19.55	0.745
12	10.0		6.428		530	1013	"	5.952	.029	.586	.060	1.066	....	....
13	10.1		6.428		525	1014	"	5.894	.029	.620	.065	1.017	11.90	.627
14	10.1		6.428		490	1013	"	5.754	.026	.568	.055	0.959	8.21	.473
						Nitrogen of Urine = 47.430 Nitrogen of Fæces = 1.845 Nitrogen of Hair = 1.059								
Totals			50.995		3962			50.334	0.248	4.856	0.562	7.519	39.66	1.845
Daily Averages			6.374		495			6.292	0.031	0.607	0.070	0.940		0.231

## THIRD EXPERIMENT. — BORIC ACID PERIOD.

DATE.	BODY.		FOOD.		BORIC ACID.		URINE.						FÆCES.			
	Weight.	kilos.	Nitrogen.	grams	Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitrogen.		
May.					c.c.		litmus									
15	10.1		6.428	1	525	1015	Acid.	5.677	0.039	0.558	0.068	1.003	2.73	0.157		
16	10.1		6.396	1	441	1015	"	5.424	.035	.627	.066	0.785	....	...		
17	10.2		6.396	1.5	401	1014	"	4.247	.053	.454	.038	0.502	....	....		
18	10.2		6.396	2	490	1015	"	5.909	.018	.637	.076	0.927	9.90	.529		
19	10.1		6.396	2.5	555	1016	"	6.934	.031	.734	.100	1.184	....	....		
20	10.2		6.396	3	465	1016	"	6.131	.041	.606	.080	0.806	9.68	.557		
21	10.2		6.396	3	400	1014	"	4.588	.034	.457	.042	0.467	....	....		
22	10.3		6.396	3	500	1018	"	7.029	.059	.689	.099	1.080	11.86	.579		
					Nitrogen of Urine = 45.939											
					Nitrogen of Fæces = 1.822											
					Nitrogen of Hair = 1.265											
Totals					3777			49.026	0.310	4.762	0.569	6.754	34.17	1.822		
Daily Averages					472			6.128	0.039	0.595	0.071	0.844		0.228		

THIRD EXPERIMENT. — SECOND AFTER PERIOD.

DATE.	BODY.		FOOD.		URINE.							FÆCES.		
	Weight.	kilos	Nitrogen.		Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitrogen.
			grams	c.c.										
1897.														
May.														
23	10.3		6.396		402	1015	Acid.	5.424	0.051	0.597	0.075	0.671	3.95	0.192
24	10.3		6.396		445	1010	Alkaline.	3.957	.028	.394	.031	0.289	....	....
25	10.1		6.410		620	1014	Acid.	7.224	.066	.787	.077	1.115	....	....
26	10.2		6.410		521	1013	"	5.730	.051	.541	.045	0.911	9.76	.542
27	10.1		6.410		550	1014	"	5.614	.039	.601	.050	0.892	....	....
28	10.1		6.410		470	1016	"	6.518	.033	.722	.066	1.103	....	....
29	10.2		6.410		455	1013	Alkaline.	4.994	.041	.549	.043	0.692	....	....
30	10.3		6.410		480	1017	Acid.	6.977	.045	.769	.065	1.113	15.04	.731
			Nitrogen of Urine = 46.438											
			Nitrogen of Fæces = 1.465											
			Nitrogen of Hair = 1.227											
Totals			51.252		3943			49.130	0.354	4.960	0.452	6.786	28.75	1.465
Daily Averages			6.406		493			6.141	0.044	0.620	0.056	0.848		0.183

## THIRD EXPERIMENT.—SECOND BORAX PERIOD.

DATE.	BODY.		FOOD.		BORAX.		URINE.						FÆCES.	
	Weight.	kilos	Nitrogen.	grams	Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitrogen.
May.					c.c.		litmus				grams			
31	10.1		6.410	10	530	1027	Alkaline.	7.711	0.053	0.836	0.070	1.474	....	....
June 1	10.1		6.410	10	420	1029	"	6.384	.037	.671	.052	1.102	....	....
2	10.3		6.410	5	360	1026	"	6.627	.029	.761	.099	0.998	19.10	0.874
3	10.4		6.410	6	342	1020	"	4.574	.029	.495	.038	0.336	....	....
4	10.3		6.392	7	540	1022	"	8.025	.042	.828	.113	1.112	....	....
5	10.2		6.374	10	450	1025	"	5.634	.031	.610	.077	0.680	19.40	1.019
6	10.2		6.374	8	502	1023	"	6.495	.040	.629	.070	1.005	....	....
7	10.3		6.374	8	513	1023	"	6.913	.034	.683	.076	0.809	17.55	0.844
Nitrogen of Urine = 52.363 Nitrogen of Fæces = 2.737 Nitrogen of Hair = 0.932														
Totals	.	.	51.154	64	3657			56.032	0.295	5.513	0.595	7.516	56.05	2.737
Daily Averages			6.394	8	457			7.004	0.037	0.689	0.074	0.939		0.342

THIRD EXPERIMENT.—THIRD AFTER PERIOD.

DATE.	BODY.		FOOD.	URINE.						FÆCES.			
	Weight.	Nitrogen.	grams	Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitrogen.
1897.													
June.						litmus					grams		
8	10.3	6.374	411	1016	Acid.	6.213	.042	.567	.033	.644	....	....	....
9	10.3	6.374	525	1013	"	5.834	.029	.558	.043	.686	....	....	....
10	10.4	6.374	422	1011	"	4.284	.042	.447	.047	.399	....	....	....
11	10.4	6.374	500	1014	"	6.149	.037	.638	.058	.872	....	....	....
12	10.4	6.374	525	1016	"	7.560	.051	.781	.075	1.344	22.80	0.895	0.895
13	10.3	6.409	503	1013	Alkaline.	5.158	.043	.518	.030	.644	....	....	....
14	10.2	6.445	652	1015	Acid.	7.917	.040	.856	.082	1.445	....	....	....
15	10.2	6.445	512	1012	"	5.663	.044	.631	.065	.793	20.06	1.194	1.194
						Nitrogen of Urine = 48.778 Nitrogen of Fæces = 2.089 Nitrogen of Hair = 0.963							
Totals . . .			51.169	4050			51.830	0.328	4.996	0.433	6.827	42.86	2.089
Daily Averages			6.396	506			6.479	0.041	0.624	0.054	0.853		0.261

## THIRD EXPERIMENT.—GENERAL SUMMARY.

PERIODS.	TOTAL NITROGEN.			URINE.							FACES.		HAIR.
	Ingested.	Excreted.	Balance.	Vol.	Nitrogen.	Uric Acid.	Total SO <sub>3</sub> .	Combined SO <sub>3</sub> .	Total P <sub>2</sub> O <sub>5</sub> .	Dry Weight.	Nitrogen.	Nitrogen.	
grams				c.c.	grams								
Normal . .	52.163	51.734	+ 0.429	4196	49.093	0.315	4.515	0.495	7.738	24.69	1.417	1.224	
Borax. . .	50.885	51.686	— 0.801	3761	48.324	.293	4.905	.662	7.374	43.75	2.176	1.186	
After . . .	50.995	50.334	+ 0.661	3962	47.430	.248	4.856	.562	7.519	39.66	1.845	1.059	
Boric Acid .	51.200	49.026	+ 2.174	3777	45.939	.310	4.762	.569	6.754	34.17	1.822	1.265	
After . . .	51.252	49.130	+ 2.122	3943	46.438	.354	4.960	.452	6.786	28.75	1.465	1.227	
Borax. . .	<b>51.154</b>	<b>56.032</b>	<b>— 4.878</b>	<b>3657</b>	<b>52.363</b>	<b>.295</b>	<b>5.513</b>	<b>.595</b>	<b>7.516</b>	<b>56.05</b>	<b>2.737</b>	0.932	
After . . .	51.169	51.830	— 0.661	4050	48.778	.328	4.996	.433	6.827	42.86	2.089	0.963	



Throughout the entire experiment of fifty-six days the animal remained perfectly well, kept a fairly constant body-weight, and showed no symptoms of nausea or vomiting during the administration of either borax or boric acid. The only noticeable effect was a seeming loss of appetite on one day, as mentioned above. At the termination of the final after period, a single dose of 5 grams of boric acid was given. This resulted in vomiting 4-5 hours afterward.

The relative excretion of nitrogen for the seven periods is shown in the following table:

	1. <i>Fore Period.</i>	2. <i>First Borax Period.</i>	3. <i>First After Period.</i>
Nitrogen of Food . . . .	52.163	50.885	50.995
Nitrogen of Urine . . . .	49.093	48.324	47.430
Nitrogen of Fæces . . . .	1.417	2.176	1.845
Nitrogen of Hair . . . .	1.224	1.186	1.059
	51.734	51.686	50.334
Nitrogen Balance . . .	+ 0.429	- 0.801	+ 0.661
Ratio of Urine and Hair Nitrogen to Food Nitrogen .	96.4 per cent.	97.2 per cent.	95.0 per cent.
	4. <i>Boric Acid Period.</i>	5. <i>Second After Period.</i>	6. <i>Second Borax Period.</i>
Nitrogen of Food . . . .	51.200	51.252	51.154
Nitrogen of Urine . . . .	45.939	46.438	52.363
Nitrogen of Fæces . . . .	1.822	1.465	2.737
Nitrogen of Hair . . . .	1.265	1.227	0.932
	49.026	49.130	56.032
Nitrogen Balance . . .	+ 2.174	+ 2.122	- 4.878
Ratio of Urine and Hair Nitrogen to Food Nitrogen .	92.2 per cent.	93.0 per cent.	104.1 per cent.
	7. <i>Third After Period.</i>		
Nitrogen of Food . . . .	51.169		
Nitrogen of Urine . . . .	48.778		
Nitrogen of Fæces . . . .	2.089		
Nitrogen of Hair . . . .	0.963		
	51.830		
Nitrogen Balance . . .	- 0.661		
Ratio of Urine and Hair Nitrogen to Food Nitrogen .	97.2 per cent.		

In the first borax period of eight days with a total consumption of 32.5 grams of borax, *i. e.*, an average of 4 grams per day, there is practically no change in the rate of proteid metabolism. There is, however, a slight rise in the amount of faecal nitrogen similar to that noticed in the first experiment with borax, by which the nitrogen

balance is somewhat changed, but there is plainly no effect produced on proteid metabolism. In the second borax period, on the other hand, there is evidence for the first time of a distinct and unquestionable influence upon proteid metabolism. In this period of eight days 64 grams of borax were administered, and under its influence the excretion of nitrogen through the urine was greatly increased. As in the other experiments, the proportion of nitrogen in the fæces was likewise increased, implying decreased assimilation of proteid food, but the nitrogen balance of  $-4.878$  is mainly due to direct stimulation of proteid metabolism. When, however, it is considered that to accomplish this result a daily dose of 8 grams of borax was required, and for eight consecutive days, with a dog weighing only 10 kilos, it is very plain that proteid metabolism is not readily affected by borax.

In the boric acid period of eight days, with a total dosage of 17 grams of the acid, there is some evidence of diminished proteid metabolism. The excretion of nitrogen through the urine is certainly diminished; there appears to be a sparing of proteid, but it is to be noticed that, in the period following, the nitrogen balance remains unaltered, which fact casts some doubt upon the assumption that the result is due solely to the acid. It is of course possible that the action of the boric acid may be continued into the after period, but this we should hardly expect in view of the rapid elimination of boric acid from the system. Further, after the second borax period, where the nitrogen balance is so noticeably disturbed, there is a quick return to the normal, the nitrogen balance for the final period dropping back to  $-0.661$ . Consequently, while the analytical data show a retention of nitrogen during the boric acid period, thus indicating diminished proteid metabolism, we feel some hesitation in attributing the result wholly to the boric acid, particularly as the earlier experiment with boric acid gave essentially negative results.

Especially noticeable in this experiment, as in the earlier experiment with borax, is the action of the latter agent in reducing the volume of the urine. [See table showing general summary.] In both borax periods the total volume of urine excreted is distinctly reduced, and the same holds true in this experiment with the boric acid. It is quite probable that the somewhat larger daily dose of boric acid made use of in the present experiment is responsible for this result, although it is possible of course that the personality of the animal may have had some influence. In the previous experiment

with boric acid, where the maximum daily dose was 2 grams, the volume of the urine was unaltered. In view of these facts it is perhaps proper to consider the larger dosage of boric acid used in the present experiment as responsible for the apparent action upon proteid metabolism likewise.

Also noticeable in this experiment is the influence of the larger doses of borax upon the excretion of total and combined sulphuric acid. Both of these are distinctly increased in amount during the last borax period, in harmony with the increase in proteid metabolism, and there is a suggestion of the same influence in the first borax period. Moreover, in the last borax period the excretion of phosphoric acid is noticeably increased, while the elimination of uric acid is slightly diminished. It is thus plainly evident, as already stated, that while moderate doses of borax, even long-continued, are without influence upon the nutritional processes of the body, large doses may distinctly increase the rate of proteid metabolism, giving rise not only to an increased excretion of nitrogen, but also of sulphuric acid and phosphoric acid.

In all of these experiments with borax there is constant evidence of an increase in the weight of the fæces during the borax periods. This increase in weight is due in part to an increased output of nitrogenous matter through this channel, but whether the latter is caused by diminished digestion and absorption of the proteid food or to a stimulation of the mucous or other secretions from the gastro-intestinal tract is not so clear. It has been plainly shown, however, in another connection<sup>1</sup> that while borax in moderate quantities has no inhibitory action whatever on either gastric or pancreatic digestion of proteids, larger proportions do retard the proteolytic action of both digestive fluids. Further, retardation of proteolysis with borax is much more pronounced than with boric acid; hence it seems quite probable that the increased bulk of fæces and the higher content of nitrogen therein during the borax periods is due mainly to slight retardation in the assimilation of proteid food.

Large amounts of borax likewise interfere with the assimilation of fatty foods; a statement which does not appear to be true of boric acid. In the accompanying table [page 37] are given the results of our analyses of the dry fæces, from a study of which it is plain that under the influence of large doses of borax — first and second borax

<sup>1</sup> Chittenden: Influence of Borax and Boric Acid on Digestion. *Dietetic and Hygienic Gazette*, 1893, vol. 9, p. 25.

periods of experiment third—both the total and percentage amounts of ether-soluble matter in the fæces are greatly increased. Boric acid, on the other hand, produces no such effect. In the first experiment, with borax, the evidence of decreased fat absorption is less pronounced, although both the dosage of borax and the amount of fat fed were greater than in the first borax period of experiment third. Quite possibly this apparent difference in action may be due to the personality of the animal. However this may be, it is plain that large doses of borax are prone to increase somewhat the bulk of the fæces, in part by diminishing slightly the assimilation of both proteid and fatty food, and in part, we think, through a tendency to increase the secretion of mucus. Thus, we observed in the last experiment, during the period when the largest doses of borax were given, that the fæces were more slimy than in the normal periods, and appeared to contain more mucus than ordinarily. Further, it is to be noted that under the influence of large doses of borax there is a tendency toward diarrhœa; not very marked to be sure, but sufficient to render the discharge of fæces somewhat watery.

In spite of these evidences of minor action in the intestinal tract with large doses of borax, there is no evidence whatever of any influence exerted upon intestinal putrefaction, either by borax or boric acid. Even with the largest doses of borax the combined sulphuric acid of the urine is raised rather than lowered, and careful examination of the urine daily with Jaffe's indoxyl test failed to reveal any indications pointing to an inhibitory influence exerted by either borax or boric acid upon the production of indican. If, however, one studies carefully the output of combined sulphuric acid as shown in the various tables it will be noticed that the highest figures are generally obtained on the day (or the day preceding that) on which the dog defecates; while after defecation the combined sulphuric acid of the urine falls at once. In other words, the natural obstruction of the intestine favors, as is well known, the absorption of putrefactive products, and thus leads to an increase of combined sulphuric acid in the urine. When, on the other hand, defecation occurs, the combined sulphuric acid of the urine is at once diminished in amount. Upon these natural fluctuations of combined sulphuric acid even the largest doses of borax and boric acid are without effect, not because these agents are without influence upon micro-organisms, but because they are too rapidly and completely absorbed from the intestine to exert much influence upon intestinal putrefaction. In only one in-

TABLE SHOWING CONTENT OF FAT AND OTHER ETHER-SOLUBLE MATTER IN THE FÆCES.

EXPERIMENT I.					EXPERIMENT III.				
Date.	Fæces.	Ether-soluble matter.		Period.	Date.	Fæces.	Ether-Soluble matter.		Period.
1896.	Dry weight. Grams.	Per cent.	Grams.		1897.	Dry weight. Grams.	Per cent.	Grams.	
Dec. 2	38.15	35.03	13.362	Fore	Apr. 25	14.33	28.91	4.134	
					28	10.36	29.09	3.029	
7	35.91	33.60	12.067			24.69	29.01	7.163	Normal
11	24.68	25.23	6.227						
	60.59	30.02	18.294	Borax	29	2.96	29.09	0.840	
					May 3	20.10	36.35	7.306	
16	33.25	36.51	12.140		6	20.69	37.06	7.671	
20	25.45	24.36	6.198			43.75	36.15	15.817	Borax
	58.70	31.24	18.338	After					
EXPERIMENT II.					11	19.55	36.18	7.091	
Date.	Fæces.	Ether-soluble matter.		Period.	13	11.90	23.50	2.797	
1897.	Dry weight. Grams.	Per cent.	Grams.		14	8.21	25.89	2.117	
Feb. 26	6.96	23.70	1.649			39.66	30.27	12.005	After
Mar. 2	11.90	17.88	2.128						
3	10.50	16.95	1.770		15	2.73	25.89	0.705	
5	17.30	20.82	3.602		18	9.90	33.19	3.286	
	46.66	19.61	9.149	Fore	20	9.68	25.76	2.499	
					22	11.86	24.13	2.858	
8	10.20	18.87	1.924			34.17	27.36	9.348	Boric Acid
10	9.75	17.67	1.723						
12	16.30	20.31	3.311		23	3.95	24.13	0.953	
14	11.60	20.60	2.390		26	9.76	24.20	2.372	
15	5.45	20.54	1.119		30	15.04	29.54	4.443	
	53.20	19.67	10.467	Boric Acid		28.75	27.02	7.768	After
16	5.45	20.54	1.119						
17	7.71	26.63	2.053		June 2	19.10	45.01	8.596	
19	8.82	20.28	1.789		5	19.40	39.06	7.579	
21	12.25	20.72	2.538		7	17.55	33.94	5.940	
23	10.47	20.01	2.095			56.05	39.46	22.115	Borax
25	10.90	19.31	2.105						
	55.60	21.04	11.699	After	12	22.80	39.27	8.954	
					15	20.06	29.99	6.028	
						42.86	34.96	14.982	After

stance were we able to detect any boric acid in the fæces, viz., on June 5th, at a time when the largest doses of borax were being given; and at the close of this period the boric acid reaction could be obtained with the urine only on the first day of the after period, so rapidly was the borax passed out of the body.

Lastly, attention may be called to the constant presence, in appreciable amounts, of uric acid in the urine of all the animals experimented with, in opposition to the older statements of Liebig<sup>1</sup> and others that kynurenic acid may entirely replace uric acid in the urine of the dog. Our results, so far as they extend, are thus wholly in accord with the recent observations of Solomin.<sup>2</sup> We have, however, made no attempt to determine the amounts of kynurenic acid present.

**General Conclusions.**—Moderate doses of borax up to 5 grams per day, even when continued for some time, are without influence upon proteid metabolism. Neither do they exert any specific influence upon the general nutritional changes of the body. Under no circumstances, so far as we have been able to ascertain, does borax tend to increase body-weight or to protect the proteid matter of the tissues.

Large doses of borax, 5–10 grams daily, have a direct, stimulating effect upon proteid metabolism, as claimed by Gruber; such doses, especially if continued, lead to an increased excretion of nitrogen through the urine, also of sulphuric acid and phosphoric acid.

Boric acid, on the other hand, in doses up to 3 grams per day, is practically without influence upon proteid metabolism and upon the general nutrition of the body.

Borax, when taken in large doses, tends to retard somewhat the assimilation of proteid and fatty foods, increasing noticeably the weight of the fæces and their content of nitrogen and fat. With very large doses there is a tendency toward diarrhœa and an increased excretion of mucus. Boric acid, on the contrary, in doses up to 3 grams per day, is wholly without influence in these directions.

Borax causes a decrease in the volume of the urine, changes the reaction of the fluid to alkaline, and raises the specific gravity, owing to the rapid elimination of the borax through this channel. Under no circumstances have we observed any diuretic action with either borax or boric acid. The latter agent has little effect on the volume of the urine.

<sup>1</sup> LIEBIG: *Annalen d. Chem. u. Pharm.*, Band 86, p. 125.

<sup>2</sup> SOLOMIN: *Zur Kenntniss der Kynurensäure. Zeitschr. f. physiol. Chem.*, 1897, Band 23, p. 497.

Both borax and boric acid are quickly eliminated from the body through the urine, twenty-four to thirty-six hours being generally sufficient for their complete removal. Rarely are they found in the fæces.

Neither borax nor boric acid have any influence upon the putrefactive processes of the intestine as measured by the amount of combined sulphuric acid in the urine, or by Jaffe's indoxyl test. Exceedingly large doses of borax are inactive in this direction, not because the salt is without action upon micro-organisms, but because of its rapid absorption from the intestinal tract.

Borax and boric acid, when given in quantities equal to 1.5-2.0 per cent of the daily food are liable to produce nausea and vomiting.

Owing to the rapid elimination of both borax and boric acid, no marked cumulative action can result from their daily ingestion in moderate quantities.

At no time in these experiments was there any indication of abnormality in the urine; albumin and sugar were never present.

# VARIATIONS IN DAILY ACTIVITY PRODUCED BY ALCOHOL AND BY CHANGES IN BAROMETRIC PRESSURE AND DIET, WITH A DESCRIPTION OF RECORDING METHODS.\*

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IN a series of papers dealing with the laws of growth Minot<sup>1</sup> has pointed out the significance of experiments on organisms as individual wholes, as leading toward the proper object and final purpose of biological investigation,—the discovery of the laws of life. Growth, anabolic and accumulating, has its reverse in all forms of katabolism, of which by far the most important are the forms which supply from day to day and from hour to hour energy for those bodily functions which may be summed up under the broad name of “activity.” If we grant that the activity of any individual organism may be an index of the sum total of its bodily conditions, then the study of the variations of that activity, and of the conditions which lead to such variations, becomes of the utmost importance.

With the commencement of such a study its difficulties begin. How is it possible to arrive at an adequate estimate of such activity? The present methods of Science can measure metabolism but exact chemical analyses would be impossible in a long series of experiments on normal animals. It becomes then clearly necessary to make many assumptions in devising a practicable method. In the research about to be described it has been assumed that the amount of muscular energy developed, in other words the amount of work

\* Acknowledgments: I wish to express my obligation to Dr. Warren P. Lombard for permission to reproduce Figure 2, to Dr. C. F. Hodge, of Clark University, and to Messrs. D. Appleton and Co., for permission to adapt Figure 7, and to Dr. Hodge, for direction and assistance throughout my work. I am deeply indebted, also, to Martin Green, Esq., of Green Hill, Worcester, Mass., whose generosity and interest in the experiments placed barometer records at my disposal at all times—and to Mr. Jonas G. Clark, the founder of Clark University, whose permanent provision for scientific investigation alone made the work possible.

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done, by any animal day by day will be an approximate expression of its susceptibility to those variable conditions which may be chosen for experimentation. But here again new obstacles arise. No animal, even under conditions which may seem to be unvaried, will spontaneously show a uniform degree of activity for any series of days. Small causes, apparently inappreciable and unmeasurable, will produce changes tending to create or to destroy that feeling of bodily well-being which accompanies the proper functioning of all component parts. In studying the effect of drugs, for example, this difficulty becomes so overwhelming that it is plain that these small causes must be investigated for themselves. Weather changes, long recognized as potent, must be studied. The effects of atmospheric pressure, atmospheric moisture, temperature, light, winds and electrical variations must be obtained by long series of observations before we can hope to arrive at a solution of our single equation with its almost indefinite number of unknown quantities.

Of the meteorological factors just mentioned, only atmospheric pressure has been taken into consideration here. The methods of the research have nevertheless been applied for the purpose of studying the effects upon activity of variations in diet, and of the administration of alcohol, with the hope of obtaining results so broad and general as to be of value in showing at least the possibilities in this comparatively new field of operation.

So much for the purpose and aims of the undertaking. A word must be said as to the selection of suitable animals for the experiments. It seemed best to choose rats and mice, because they fill as many as possible of the requirements. They are small, cheap, easily fed and cared for; and, best of all, when placed in revolving cages they spend most of their time, when not eating or sleeping, in running.

With regard to the distribution of their working periods it may be noted that the records obtained from rats show that their activity is confined entirely to the hours of the night. Beginning at from six to eight o'clock in the evening—later in summer than in winter—they are uniformly, though not continuously, active during the next eight or ten hours. Contrasted with this is the activity of the squirrel, the records of which show greater intensity, but only for an hour or two night and morning. Still another type was that of two fox-squirrels, active throughout the whole of the day and sleeping only at night. Such differences indicate that an investigation of the

comparative distribution of activity and of the relation of its intensity to its duration promises interesting and valuable results. Commencing, as did Hodge and Aikins,<sup>2</sup> with the protozoa, it might be possible to develop much that would bear closely upon theories of animal rhythm, rest and sleep.

The following section will give a detailed account of the apparatus used, and of the general methods of procedure.

**Description of Apparatus and Methods.**—The apparatus consists primarily of two parts : the cages in which the animals to be experimented upon are placed, and the various mechanisms for recording their movements in these cages. Each of the cages (see Fig. 1) used throughout the experiments on rats is cylindrical, eighteen inches long by twenty in diameter, made of fine wire netting soldered to a

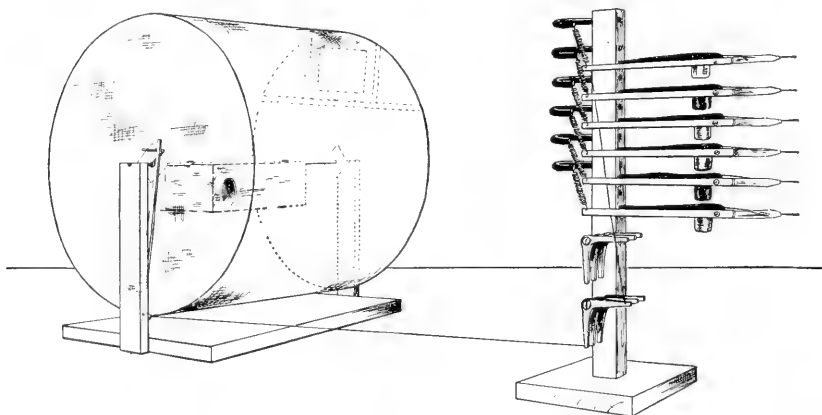


FIGURE 1.

frame of stout steel wire. The cage revolves freely on a steel rod supported by a fixed wooden frame. At one end is a wide-hinged door; from the axle is hung a light wooden nest-box, completely closed in but for a small round opening on one side; and to the end of the nest-box next the door is fixed a detachable tin feed-box of two compartments. At the opposite outer end of the cage is an eccentric which, with each revolution of the cage in either direction, pushes aside an upright lever attached to the wooden axle support, and in so doing pulls the wire connecting the cage lever with the recording apparatus.

Cages used for mice are similar in construction, except that their small size (eight inches long by five in diameter) renders them so

light that no wooden support is needed for the axle. The axle is held in position by a clamp upon an iron standard, while a second clamp holds a bearing for the lever which, with its wire, serves as a means of communicating the motions made by the cage.

To record the revolutions of the cages a simple six-inch continuous-roll kymograph, with uniform motive power, was first used. A standard carrying six light wooden levers, each five inches long, is placed before the kymograph. Each lever is tipped with thin whalebone, and to each is fixed a small glass ink-well and a pen of fine capillary glass tubing. Each is connected by a wire to the lever of the corresponding animal cage. As the cage revolves the eccentric pushes back the cage lever, the wire attached to it is pulled, the pen lever is drawn down, and the pen makes a vertical mark upon the slowly travelling scroll of paper. Upon the release of the cage lever a spring fastened to the pen draws the pen, the wires and the cage lever back to their original position, and the apparatus is ready to record another revolution of the cage.

Single revolutions would be indicated by single vertical lines, but when the cage is made to revolve with sufficient rapidity a continuous broad band marks the duration of a series of revolutions. An electro-magnetic time-marker, connected with a battery and a clock making short connections every minute and longer ones every hour, gives an accompanying record by means of which the duration and distribution of such periods of activity may be computed.

The kymograph method just described, though invaluable in the study of the distribution of activity throughout the day in different species of animals, and even in different animals of the same species, is nevertheless a rather unreliable one for close or accurate experimental measurement. After a rate of cage revolution has been reached which is sufficiently great to be recorded by a continuous broad band, no greater speed of revolution can be distinguished by any feature of the tracing. In other words, of two animals one might do twice as much work as the other in the same period of time without any indication of that fact being shown upon the record of their activity.

A much more exact recording method is the following, which has been used in almost all the experiments to be described. The hair spring and balance wheel of a common spring clock are removed, and to the escapement are attached, on one side a soft spring of fine brass wire, and upon the other a wire which, passing through a slit-

like opening in the clock case, is in turn attached, as was the wire in the preceding method, to the cage lever. Each revolution of the cage, as before, pulls the wire, the escapement is drawn down, and one cog of the ratchet wheel is let go. In the clocks used two of these cogs correspond to one second on the dial—seven thousand two hundred, therefore, to an hour. The clock is set at twelve, and the exact number of revolutions performed in any given time by the cage to which it is attached may, at the end of that time, be read off on the dial. This method fails to give any representation of the distribution of activity, but its relative accuracy in recording the total amount of work done makes it perhaps the most useful in experiments where daily variations due to food and drug effects are being sought for. By estimating the circumference of the cage used, and multiplying by the observed number of revolutions in any given time, one may obtain a rough estimate of the distance travelled.

For example, it was noted in the course of the various experiments to be described, that a common gray rat will run normally from five to fifteen miles in a single night, one particularly active rat travelling an aggregate distance of one hundred and forty-three miles in ten days.

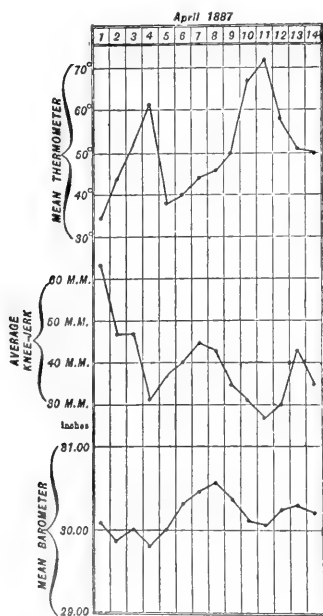


FIGURE 2. Knee-jerk, barometer and thermometer. (Lombard)

the pen slowly rises as the string is wound up. By using a roll of paper carried on a kymograph at the rate of six or eight inches a day, and recording also thermometer, barometer, hygrometer and

time, a more or less complete picture of the data for the period studied may be obtained.

**The Effect upon Activity of Changes in Barometric Pressure.**—Lombard<sup>3</sup> has described a correspondence between the variations of average knee-jerk for a series of days, and the condition of the weather. His results (see Fig. 2) show a direct relation between knee-jerk and barometric pressure, and a more indefinite inverse relation to temperature. No effect of changes in humidity, or in electric tension, was shown. Lombard<sup>4</sup> has also shown, in experiments upon himself, that a decrease in atmospheric pressure lessens the ability to do voluntary muscular work, while an increase in pressure increases muscular power. High temperature, especially when associated with much humidity, decreases this ability.

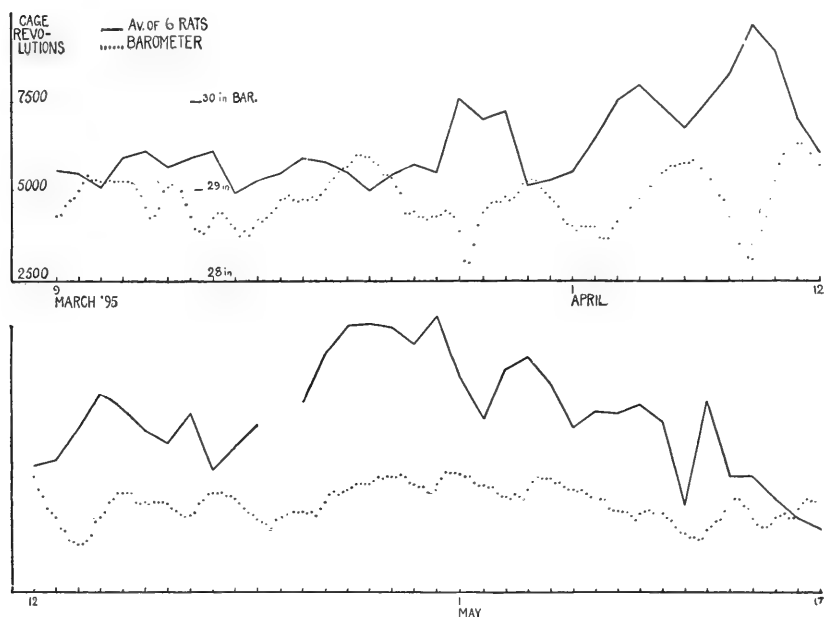


FIGURE 3. Curve of the average daily activity of six gray rats, during seventy days. The dotted line shows barometric pressure.

An undoubted influence of barometric variations upon the activity of the nerve muscle complex has, therefore, already been demonstrated, so that we might naturally expect to find variations in the amount of spontaneous daily work correlated with changes in atmospheric pressure. Turning to the experiments in which the methods

already outlined were used, we have in Figure 3 a curve, plotted in terms of cage revolutions, for seventy days, of the average daily activity of six common gray rats, full grown when caught, and therefore uninfluenced by domestication. The dotted line shows the variations in barometric pressure during the continuance of the experiment. Up to April twelfth, that is during the first half of the time, the rats were perfectly normal. During the latter half, however, four of them were getting alcohol in addition to their regular food. The consequent interference with normal activity is shown in the otherwise unaccountable rise during the latter part of April and the first few days of May. This will be referred to again more fully when the alcohol experiments are being described.

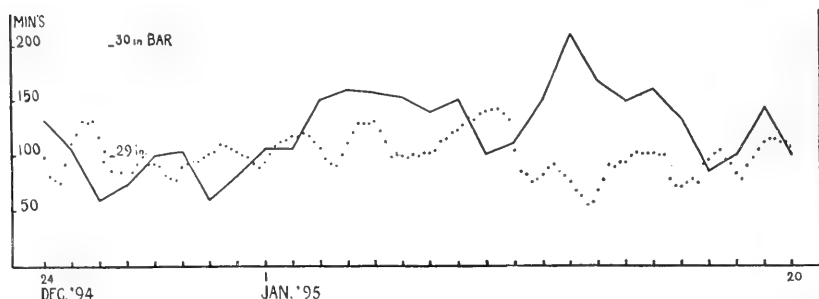


FIGURE 4. Curve of the daily activity in minutes of a single gray rat during twenty-six days. Barometer, as before, in dotted line.

Figure 4 is a curve of the variations in duration of activity shown by a single gray rat during twenty-six days. The curve of barometric pressure is superimposed in dotted line. In each of these curves there is shown an inverse relation between the amount of daily activity and the barometric pressure. Similar results were obtained from the records of a common red squirrel, though the squirrel's activity showed at times extreme variations from day to day with no apparent cause.

Figure 5 shows curves of the variations in daily activity of three groups of white rats for one hundred and twelve days. For the first twenty-six days the record is from five pairs of rats, one pair, male and female, to a cage; for the next twenty-six days six pairs are recorded, five of them being the five pairs of the preceding days; and for the rest of the time a new series of six pairs furnish the records. Where a point in the record has been enclosed in a

small circle, imperfect data have been used to fill out the space. The barometric pressure is recorded, as before, in dotted line.

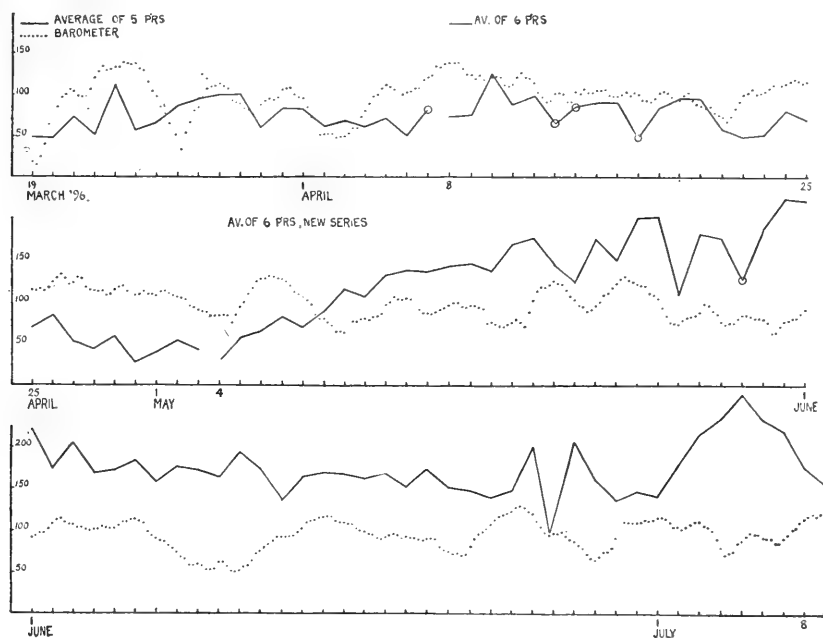


FIGURE 5. Curve of average daily activity of white rats: March 19th to April 7th, average of five pairs; April 8th to May 3d, of six pairs; May 4th to the end, of a new series of six pairs. Barometer in dotted line.

Whatever correspondence there is between the two curves would lead to the conclusion that the effect of changes in atmospheric pressure is a direct one, as against the inverse relation shown for gray rats. A point worthy of note, however, is that just as the pressure effect is shown more clearly in the curve of the first experiment where the animals are perfectly normal (see Fig. 3), so in this curve, from the beginning to the point marked April eighth, and from May fourth to June first, where the records are from normal rats, a correspondence is more clearly shown. During the rest of the time the same animals were being used for alcohol experiments, with a consequent interference as yet unexplained. Many of the daily variations in the curve are unaccounted for, but the great rise in average activity shown during the early part of July is without doubt due to the disturbing noises incident to the celebration of Independence Day.

Figure 6 gives another curve of the average activity of six white rats, with barometer plotted in dotted line as before. Here again the correspondence between the curves is only of doubtful value.

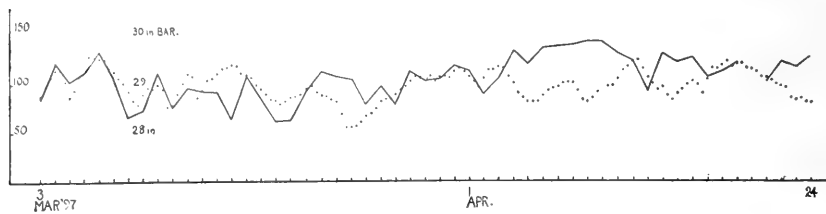


FIGURE 6. Average activity of six white rats for fifty-three days. Barometer in dotted line.

Figure 7 shows, in solid and broken line, the curves of activity of two dogs, from data obtained by Hodge<sup>5</sup> by means of a pair of ingeniously contrived pedometer watches carried by the dogs in their collars. The curves are plotted from the readings of these watches taken once a day. To the chart I have added in dotted line the curve of barometric pressure for the forty-six days of the experiment, with the result of establishing a somewhat striking correspondence. The curve would be improved in legibility if the records for the two dogs were averaged instead of being plotted

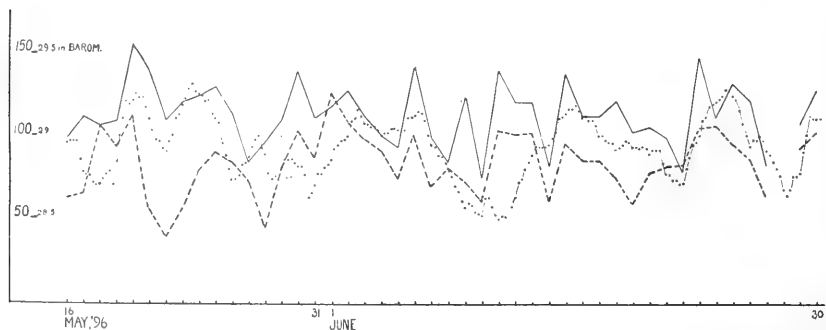


FIGURE 7. Curves of the activity of two dogs, plotted in solid and broken line. Barometric variations shown in dotted line for the forty-six days of the experiment.

separately, as they are in the figure; but it would not show, as it does now, the remarkable similarity between the two. Other measurable factors are undoubtedly at work in producing variations,—factors which can be determined only by manifold repetition of such experiments.



The foregoing charted results show an inverse relation between the amount of voluntary daily activity of gray rats and atmospheric pressure, and a direct relation between variations in barometric pressure and the activity of the two dogs. The white rats experimented upon showed only a doubtful direct relation between the amount of their daily activity and pressure. The experiments seem to point to the possibility of a fundamental difference in this respect between domesticated animals, independent of weather changes, and wild animals with their greater need of individual effort for self-preservation and their greater interest in food supply.

**The Effect of Changes in Diet.** — The experiments under this head were all upon white rats normally fed on a uniform diet of dog-biscuit and water. In the first experiment (see Fig. 8) six pairs of white rats, one pair to a cage, were used. The solid line shows the average daily activity of three pairs, the broken line that of the other three.

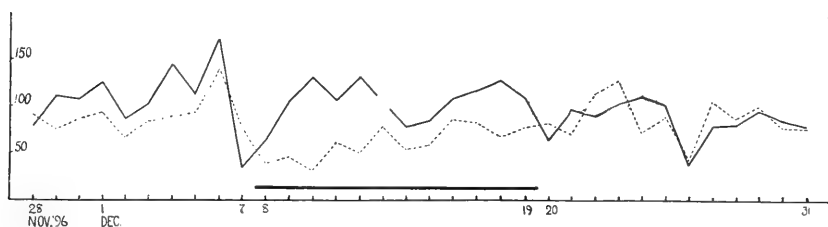


FIGURE 8. Feeding experiment. Each line shows a curve of the daily average of three pairs of white rats. From Dec. 8th to 19th those of the dotted line get beef and dog-biscuit, the others corn. For the rest of the time all were fed dog-biscuit.

Both groups were normal up to December seventh; then those represented by the broken line were fed fat and lean raw beef and dog-biscuit for twelve days, during which time the others got Indian corn. From the twentieth of December to the end of the experiment, twelve days again, all were fed dog-biscuit as before. The decrease in voluntary activity with the heavier diet is shown in the curve.

In the second experiment (see Fig. 9) all six pairs, the same rats as were used in the preceding experiment, were fed alike. For the first twelve days they were fed on dog-biscuit, with good average records as the result. During the next fifteen days (January first to fifteenth) white bread was given, with an accompanying increase in the amount of daily work. For the next ten days (January sixteenth to twenty-fifth) bread and fat and lean beef were given, causing a manifest decrease. Then for six days (January twenty-sixth to

thirty-first) bread alone, as before, was fed, with again a rise. This was followed by bread and beef for seven days (February first to seventh) with a fall in activity. From the eighth of February to the end of the experiment on the first of March bread alone was fed,

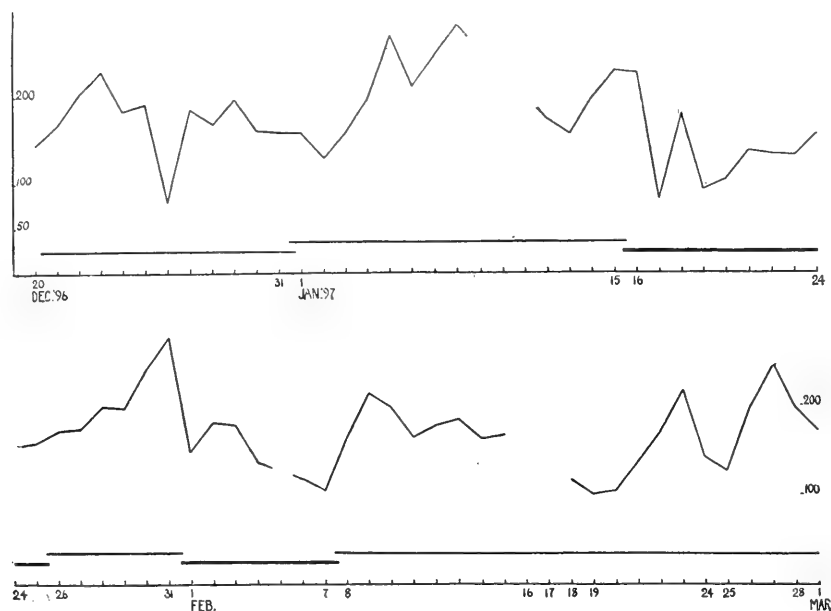


FIGURE 9. Second feeding experiment. Curve shows average activity of six pairs of white rats. December 20th to 31st — dog-biscuit. January 1st to 15th — bread. January 16th to 25th — beef and bread. January 26th to 31st — bread. February 1st to 7th — beef and bread. February 8th to March 1st — bread. At points marked February 18th and 19th the effect of lack of food is shown; at February 24th and 25th, the effect of an escape of gas, and the same at March 1st.

with a resulting rise in activity. The record for this last period remains uncomplicated, however, during only the first eight days. On the seventeenth of February the record for the preceding night was not noted, nor were the rats fed. The records for the sixteenth and seventeenth were therefore lost, while the records for the eighteenth and nineteenth show the effect of insufficient food. Recovery was complete by the twenty-third, when unfortunately gas escaped in the room in which the experiments were carried on, and several of the rats were noticeably poisoned by it — the effect upon their activity being shown by the records of the twenty-fourth and twenty-fifth. Re-

covery again followed, when, on the twenty-eighth, gas escaped once more, with a similar result.

The next figure shows in another way the result of this experiment (see Fig. 10). The average for each of the periods mentioned has been taken from the perfect records of that period, while the average activity for the whole time is also shown by a horizontal broken line.

For the third feeding experiment (see Fig. 11) six male rats were selected, and all were fed on dog-biscuit for the first ten days. Then from the thirteenth to the thirtieth of March the three whose average activity is plotted by the broken line were fed beef, cheese, sugar, chocolate, and bread, while the other three, the solid line, got bread alone. From the thirty-first of March to the nineteenth of April the conditions were reversed, those previously well fed getting the meagre diet of bread. During the last five days the conditions were again reversed, with a

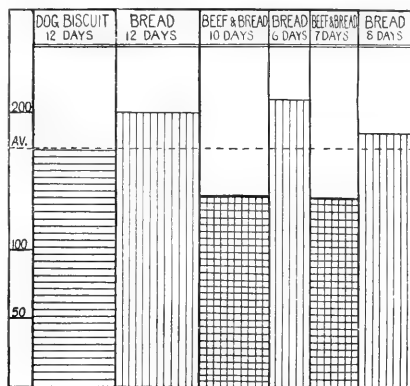


FIGURE 10. Another rendering of Figure 9, showing graphically the average activity for each of the periods of the second feeding experiment.

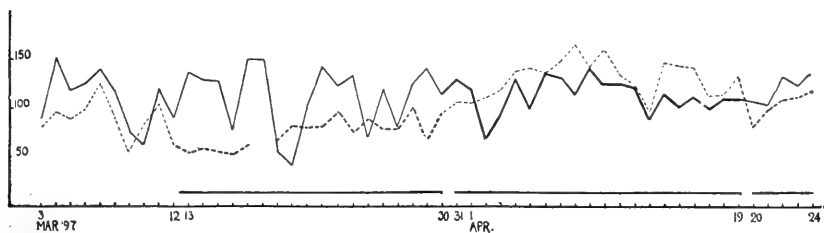


FIGURE 11. Third feeding experiment. Each line the average of the records of three male rats. All normal to March 12th. March 13th to 30th, full diet for those of the broken line and bread for the others. March 31st to April 19th, full diet for those of the solid line, and bread for the others. April 20 to 24th, the conditions again reversed.

second crossing of the lines of activity as the result. The simpler diet in all cases gives a relatively greater degree of activity. The weights of the rats were taken at the commencement of the experiment, at each change, and at the end. The following table shows the increase in weight that accompanied the decrease in activity.

Rat No.	March 3d. Commencement.	March 31st. After rich diet.	April 20th. After diet of bread.	April 25th. After rich diet.
1	305 g.	329 g.	310 g.	325 g.
2	312	335	322	325
3	235	249	238	240
	Commencement.	After diet of bread.	After rich diet.	After diet of bread.
4	315 g.	307 g.	332 g.	326 g.
5	300	281	286	280
6	260	231	256	255

**Effect of Alcohol.**—Hodge,<sup>5</sup> in the published account of his experiments on the physiological effect of alcohol on dogs, records the observation that the two alcohol dogs, although never intoxicated, show much less activity than the two normal controls. The use of a pair of pedometer watches developed the fact that, of the males, the alcoholic showed during forty-six days only seventy-one per cent of the activity of the other, while with the females the alcoholic showed a percentage of only fifty-seven.

In the first series of my own experiments gray rats were used. Six rats of as nearly equal weights as could be found were placed in six similar cages and kept on a normal and entirely uniform diet for longer or shorter periods before the actual commencement of the experiment. Averages of their activity for such periods were taken on April twelfth, 1895, and the rats were arranged in pairs in such a way that the total average activity of any one pair was as nearly equal to that of either of the other two pairs as possible. It was decided to give weak alcohol to two, strong alcohol to two, and to keep the other two as normal controls. Alcohol was administered with their food in increasing strength, according to the following schedule:

	April 12th.	15th.	22d.	27th.	May 2d.	6th.	10th.	14th.
Normals . . .	0%	0%	0%	0%	0%	0%	0%	0%
Weak . . .	5	10	15	20	—	—	—	—
Strong . . .	5	10	15	20	30	40	50	60

So that one pair from April twenty-seventh to the end of the experiment in October of the same year drank nothing but twenty per cent alcohol, while another pair had a sixty per cent solution after May fourteenth.

Figure 12 shows the voluntary activity of these six rats during the experiment. The solid line is the curve of the average of the two normal rats, the dotted line that of the weak alcohol rats, and the

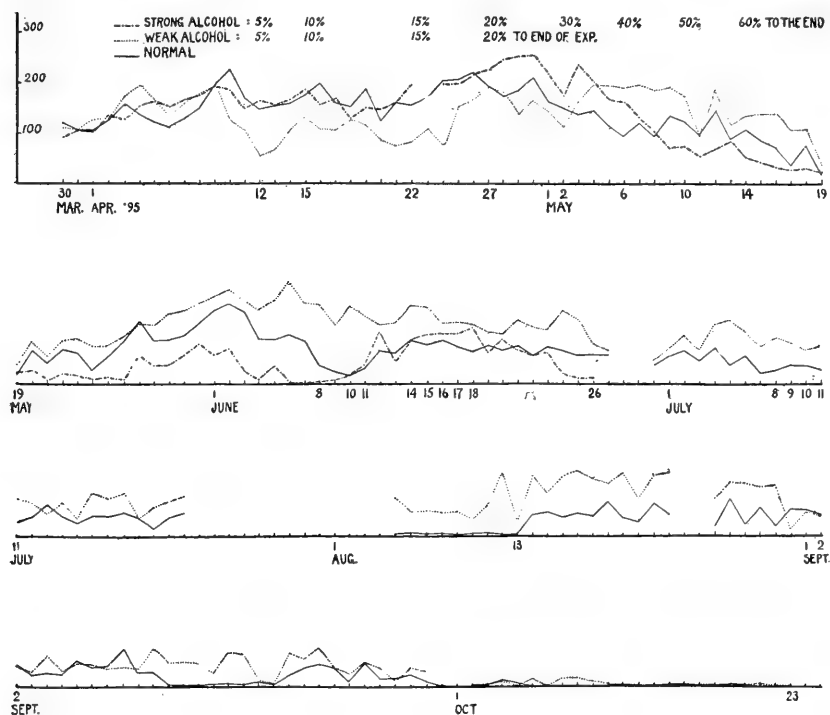


FIGURE 12. Alcohol experiment upon gray rats. Solid line plots the average of two normal rats; dotted line, of two getting weak alcohol; and broken line, of two getting strong alcohol. Water given June 8, 10, 11, 14, 15, 16, 17 and 18, and July 8, 9 and 10.

broken line, that of the strong. One of the rats getting the strong solution died on the seventeenth of May, the second on the twenty-sixth of June. On the eighth, tenth, eleventh, fourteenth, fifteenth, sixteenth, seventeenth and eighteenth of June, and on the eighth, ninth and tenth of July, water was substituted for alcohol. The result was a decided rise in the activity of the strong alcohol rats and no definite effect upon the weak alcohol animals. The

general falling off in activity toward the end of the experiment is no doubt partly due to the lack of variety and general insufficiency in diet.

Figure 13 gives another rendering of the same curve, with the normals plotted as a level line and the others shown as varying above and below that normal. The broken line is the curve of the strong

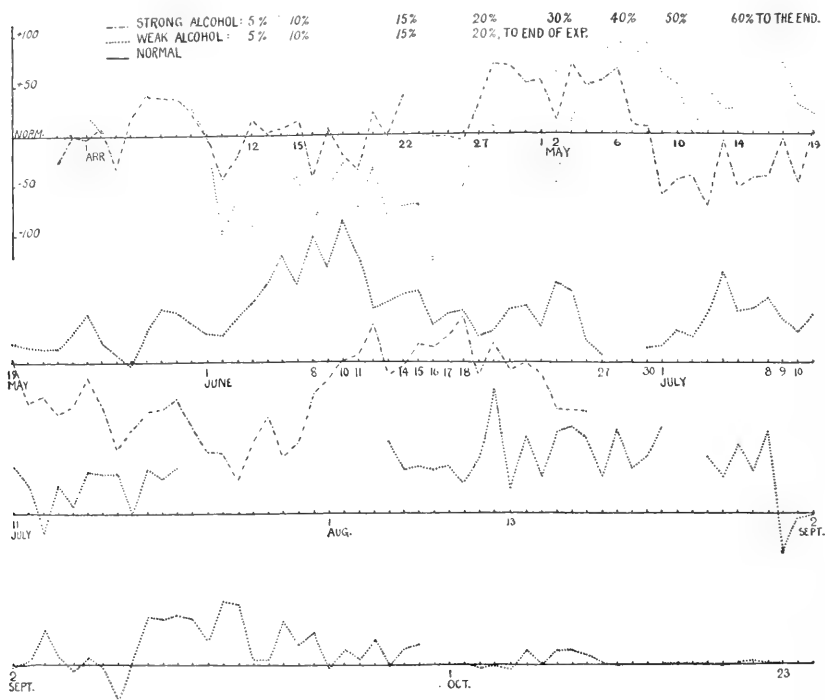


FIGURE 13. Another chart of the same experiment. Activity of normal rats taken as base line; strong alcohol rats in broken line, and weak alcohol rats in dotted line. Water given June 8, 10, 11, 14, 15, 16, 17 and 18, and July 8, 9 and 10.

alcohol rats, and the dotted line that of the weak. It shows perhaps better than Figure 12 the primary rise in the activity of the rats getting strong alcohol, with a subsequent fall as soon as forty per cent is given, and the rise again during the month of June when water was substituted. For the animals getting weak alcohol it shows a slower rise but no subsequent fall to the level of the normals for any length of time.

Figure 14 is a chart showing the result of an experiment upon six pairs of white rats, one pair, male and female, to a cage. Three

pairs are normal throughout, their average daily activity being plotted in solid line. The other three are normal for twenty-nine days, until June second, when five, ten, fifteen and twenty per cent alcohol are given on four successive days, the twenty per cent alcohol

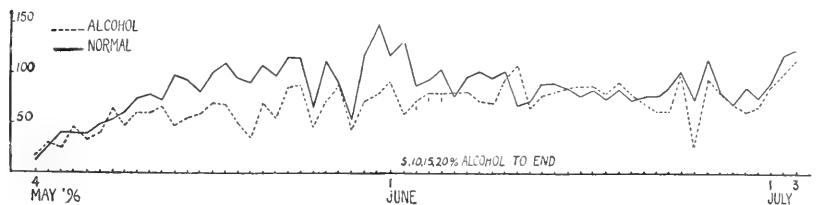


FIGURE 14. Alcohol experiment on white rats. Each line shows the average of three pairs — all normal until June first. Those shown in dotted line are then fed alcohol; the others, the solid line, are control animals.

being continued until the end of the experiment on July third. The curve shows no decrease as the result of administering alcohol in twenty per cent solution, but rather a relative increase. This curve, too, gives an example of increase through practice — a curve of getting used to the apparatus.

Figure 15 shows, for another experiment, a decrease in activity following immediately upon the administration of thirty per cent

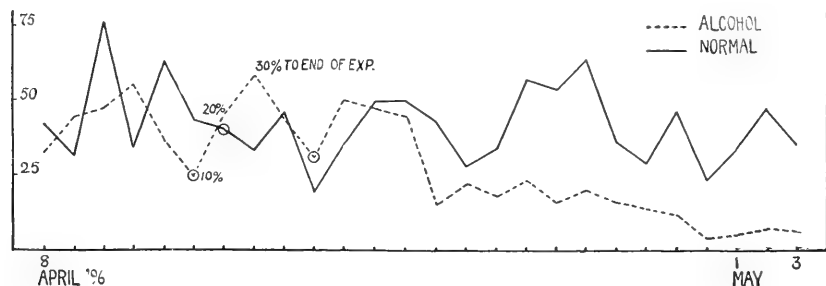


FIGURE 15. Alcohol experiment on white rats. Each curve shows the average of three pairs. The solid line shows the activity of the normal animals, the broken line that of the alcoholic animals. Alcohol given as indicated. Points enclosed in small circles are filled in from imperfect data.

alcohol. As in the preceding experiment, six pairs of white rats, three for alcohol and three for controls, were used. Points enclosed in circles are filled in from imperfect data.

## CONCLUSIONS.

1. While the experiments show, for gray rats and for a red squirrel, an influence of barometric pressure on voluntary, spontaneous activity that indicates an inverse relation between the two, the curves for dogs, and possibly also for white rats, show a direct effect of atmospheric pressure in increasing voluntary activity. The difference may be due to a constant difference between domesticated and wild animals.

2. Influences other than barometric pressure are undoubtedly at work to produce variations which must be considered as normal.

3. The effect of a rich diet upon white rats is to decrease voluntary activity, while that of a plain though apparently sufficient diet is to increase it. This increase, in one experiment, has been correlated with a slight loss in weight, while the decrease was accompanied by a corresponding gain.

4. The activity of rats is markedly decreased by the administration of alcohol in thirty to sixty per cent solutions, but no decrease has been experimentally demonstrated as a result of giving a twenty per cent solution of pure alcohol.

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# THE INFLUENCE OF HIGH ARTERIAL PRESSURES UPON THE BLOOD-FLOW THROUGH THE BRAIN.

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THE conditions controlling the circulation of blood in the brain are peculiar, and offer an intricate physical problem the solution of which has been attempted from an experimental as well as from a purely theoretical standpoint. The fact that the brain is contained within a rigid box that does not permit free expansion of the organ, has led some authors to assume that dilatation of its arteries, however produced, is not followed by an increased flow of blood, as is usual in other organs, but on the contrary, under certain conditions at least, by a lessened flow. Thus, Geigel<sup>1</sup> has held upon theoretical grounds that dilatation of the arteries of the brain is accompanied by a compression of the capillaries, owing to the fact that the expansion of the arteries causes an increase in intracranial pressure that is transmitted to the capillaries. Upon this view, therefore, dilatation of the arteries, whether produced through vaso-dilator nerve fibres or by a rise in general arterial pressure, should be followed by a lessened flow of blood through the brain, and *vice versa*.

Grashey<sup>2</sup> in his well-known treatise upon the hydro-statics and hydro-dynamics of the cerebral circulation has also admitted a similar possibility. Grashey assumes that intracranial pressure depends upon two conditions, namely, the volume of the cerebro-spinal liquid and the amount of arterial pressure. Dilatation of the arteries must lead to an increased intracranial pressure, and this being transmitted through the brain substance acts upon the part of the circulation where the intravascular pressure is least, that is, the cerebral veins. Inasmuch as the pressure within the capillaries is greater than in the veins, an increase of intracranial pressure should affect the veins and not the capillaries, as Geigel assumed. Moreover, Grashey emphasizes a fact that other authors seem sometimes to overlook, namely, that the sinuses of the brain are probably entirely protected from any direct influence of intracranial pressure by their tense and inextensible covering of dura mater. From his theoretical standpoint Grashey concludes that an increase in arterial pressure causes a greater flow

of blood through the brain up to a certain limit only. So soon as intracranial pressure is raised by the expansion of the arteries to such an extent as to cause occlusion of the cerebral veins, the volume of blood circulating through the brain is diminished. There follows under these circumstances what Grashey calls a vibration of the veins. The occlusion of the veins by the intracranial pressure is overcome by the consequent rise of static pressure transmitted through the capillaries, and this in turn is followed by occlusion as the pressure in the patent veins falls, and so on.

A similar view has been held by other authors who have treated the subject from a theoretical standpoint, and by some of those who have investigated the matter experimentally. The differences of opinion seem to be mainly as to the level of arterial pressure at which an obstruction to the blood-flow occurs, whether it falls within the range of normal variations of pressure or is reached only under extraordinary conditions. This admission appears to be of the nature of a theoretical concession to the recognized peculiarities of the cerebral circulation, and it is of interest to inquire how far it is supported by actual experiments. The simple and direct method of solving the problem is to measure the outflow of venous blood from the brain under different conditions of arterial pressure. This method has been employed by a number of authors with results which seem to be uniform and quite opposed to the conclusion obtained from a theoretical consideration alone. The experiments carried out by Gaertner and Wagner,<sup>3</sup> and since practically repeated by Bayliss and Hill,<sup>4</sup> Hill and Nabarro,<sup>5</sup> Reiner and Schnitzler<sup>6</sup> and others, have shown conclusively that the flow of blood through the brain is increased as the general blood pressure is raised. Even maximal blood pressures produced by the action of strychnine or absinthe give only an increased outflow from the cerebral veins with no indication of even a temporary slowing.

While these experiments have shown quite conclusively that a rise of blood pressure, so far as it can be produced by the normal regulating mechanisms of the circulation, fails to produce the condition of a diminished blood-flow as demanded by theory, it is held by some of the authors quoted that if the arterial pressure be still further increased, a point must be reached at which, owing to the compression of the cerebral veins, a permanent or temporary diminution of blood-flow will result. Thus Hill<sup>7</sup> says, "It is, however, possible that a very sudden and abnormally high rise of arterial pressure should so expand

the arteries at the base of the brain as to temporarily express capillary areas and produce anæmia."

It has been the object of my experiments to determine whether or not this is true. For this purpose I have used the simplest possible method. Upon dogs I have connected the arteries of the brain with reservoirs of blood, or Ringer's solution isotonic with the blood, that could be placed at any desired height, while the outflow from the brain was caught in a large test tube suspended by a spiral spring so that its movement, as it filled with blood, could be registered upon a kymographion. The details of the experiments were as follows: The animal was bled to death under ether. The internal carotids were dissected out and ligated. The vertebral arteries were exposed, and cannulas filled with defibrinated blood or isotonic salt solutions were introduced. These cannulas were connected with the reservoirs of carefully filtered calf's blood or Ringer's solution. In some cases the inflow cannulas were placed in the aorta or subclavians instead of in the vertebrals, and the flow directed into these latter arteries by ligating the other branches. To catch the outflow of blood from the brain, cannulas were placed directly in the superior cerebral veins at their emergence from the skull, or in some cases in the external jugular vein after ligating all communicating branches except the two superior cerebral veins. The internal jugular veins on the two sides were ligated close to the skull. An attempt was also made in some cases to shut off the outflow through the occipital sinuses into the spinal plexuses, but as this involved some danger of altering the conditions of pressure within the skull the attempt was abandoned. In the experiments as made, therefore, two paths of exit were opened to the blood flowing from the brain,—one, which was not measured, into the spinal plexuses, and one through the transverse sinuses and superior cerebral veins. The outflow from the latter was measured. This sufficed for the purposes of the experiment, inasmuch as in the dog this latter path is the one through which most of the blood escapes, and the object of the experiment was simply to determine the effect of very high arterial pressures upon the rate of outflow from the sinuses. The outflow cannulas from the two sides were united to a single short tube by means of a Y piece, and this tube opened into the test tube mentioned above. This test tube was provided below with an opening through which it could be emptied rapidly when desired. The tube was swung by a spiral of German silver wire after the manner sug-

gested by Bowditch for plethysmographic records, the spiral being so adjusted that the level of the liquid in the test tube remained constant. The movements of the tube as it filled with blood were recorded upon a kymographion upon which also a time record in seconds was taken simultaneously. As the movement downward of the test tube was constant and could be calibrated beforehand, data were obtained for calculating exactly the volume and the velocity of the outflow from the brain.

In performing an experiment two reservoirs were connected with the inflow cannulas; one of these was placed at a sub-normal level of from 30 to 60 mm. of mercury, while the other was at a height sufficient to cause a pressure of from 300 to 500 mm. Hg. The pinchcocks connected with the lower reservoir were first opened and the rate of flow was determined at this level. This pressure was then changed suddenly to the higher level by opening the pinchcock connected with the upper reservoir and closing the one on the tubing from the lower reservoir. The pressure was then brought back to the original amount by again making connections with the lower reservoir. The experiment was usually repeated a number of times. So long as the pressure was kept low, the rate of out-flow remained constant or nearly constant for some time, but exposure to the very high pressures brought about quickly an œdematous condition of the brain which diminished markedly the total outflow or might even suppress it entirely when the arterial pressure was subsequently lowered.

The direct results of the sudden change from sub-normal to supra-normal blood pressures were, however, the same in all cases; the venous outflow was increased at once to a proportional amount, and there was never any indication on the record of even a temporary blocking of the flow through the brain. The circulation through the brain under these conditions behaves in fact precisely as it does in the other organs of the body that are not enclosed in rigid cases.

The nature of the results obtained are indicated by the accompanying figures (Figs. 1 and 2) which are reproduced from the curves of two of the experiments. Examples of the actual amounts of outflow as calculated from the records are as follows:—

*Exp. 1.* Small dog — bled to death — blood defibrinated (140 c.c.) and mixed with equal volumes of normal saline and Ringer's solution to make 4 litres. Inflow cannulas placed in the vertebrals, outflow cannulas in the superior cerebral veins just at their emergence from the skull.

Arterial pressure of	30	mm. mercury	=	outflow of	7.02	c.c. per min.
"	"	" 60	"	"	18.03	" " "
"	"	" 380	"	"	102.66	" " "
"	"	" 60	"	"	10.24	" " "

2d series of observations on the same animal.

Arterial pressure of	60	mm. mercury	=	outflow of	9.65	c.c. per min.
"	"	" 400	"	"	85.14	" " "
"	"	" 60	"	"	5.85	" " "

*Exp. 2.* Small dog — bled to death from carotid — blood defibrinated (130 c.c.) and mixed with equal volumes of Ringer's solution and normal saline to make 5 litres. Inflow cannulas placed in the vertebrals, outflow cannulas in the external jugulars after ligating all branches except the superior cerebrals.

Arterial pressure of	30	mm. mercury	=	outflow of	5.26	c.c. per min.
"	"	" 122	"	"	34.52	" " "
"	"	" 30	"	"	2.22	" " "

2d series of observations on the same animal.

Arterial pressure of	50	mm. mercury	=	outflow of	2.57	c.c. per min.
"	"	" 375	"	"	70.2	" " "
"	"	" 50	"	"	1.17	" " "

*Exp. 3.* Large dog — bled to death from the carotids. For irrigating used freshly defibrinated blood of young calves filtered first through a single layer of muslin and then through four layers of the same. Inflow cannulas in the vertebrals, outflow cannulas in the superior cerebral veins at their emergence from the skull. Mercury manometers were also connected with the torcular and with the sub-dural space at the parietal eminence through trephine holes in the skull.

Arterial pressure of	60	mm. mercury	=	outflow of	23.4	c.c. per min.
A second determination	5	min. later	=	"	22.23	" " "
Arterial pressure of	335	mm. mercury	=	"	231.66	" " "
Rise of pressure in torcular cannula	36	mm. of mercury.				
"	"	" cannula in sub-dural space	=	30	mm. mercury.	

Arterial pressure of	60	mm. mercury	=	outflow of	14.66	c.c. per min.
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2d series of observations on the same animal — the flow meanwhile had diminished greatly, owing to leakage.

Arterial pressure	60	mm. mercury	=	outflow of	1.74	c.c. per min.
"	"	460	"	"	252.72	" " "

Pressure in torcular increased 52 mm. mercury.

" " sub-dural space increased 20 mm. mercury. The want of correspondence between the intracerebral and torcular pressures was evidently due to the brain being forced into the trephine hole in the case of the former, thus blocking off the manometer.

*Exp. 4.* Small dog — bled to death from carotid. Irrigation liquid was blood of young calves carefully filtered. This blood had been kept over night and had frozen. This fact probably accounts for the unusually rapid diminution in flow as the experiment proceeded, the red corpuscles not passing readily through the capillaries and clogging them. Inflow cannulas in the subclavians, ligatures being so placed as to leave an open path only to the vertebrals; outflow cannulas in the superior cerebral veins at their emergence from the skull.

Arterial pressure 60 mm. mercury = outflow not measurable with accuracy.

" " 190 " " = " of 28.08 c.c. per min.

" " 320 " " = " " 47.38 " " "

2d series on the same animal.

Arterial pressure of 60 mm. mercury = outflow not measurable with accuracy.

" " " 400 " " = " of 42.12 c.c. per min.

" " " 500 " " = " " 50.02 " " "

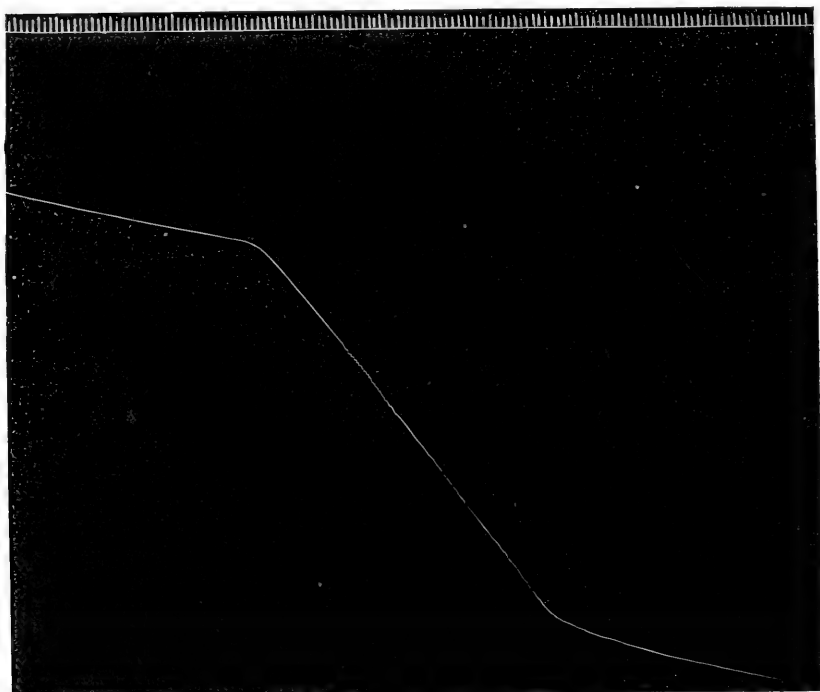


FIG. 1. Record of venous outflow from brain under arterial pressures of 60 mm., 380 mm., and 60 mm.

Under 60 mm. Hg. the outflow = 18.13 c.c. per min.

" 380 " " " " = 102.66 " " "

Return to 60 " " " " " = 10.24 " " "

The time record at the top of the illustration is in seconds.

It is evident from the data given that in all the experiments made, the blood-flow through the brain diminished as the experiment proceeded, and this effect was most marked after the blood vessels had been submitted to very high pressures. The probable explanation of this fact is that the dead capillary walls permitted a rapid filtration of liquid, which rendered the brain oedematous. This condition, in-

deed, was apparent to the eye when the brain was exposed after submission to the high intravascular pressures. This variation from normal conditions does not, however, affect the value of the experiments so far as the main point under investigation is concerned. The

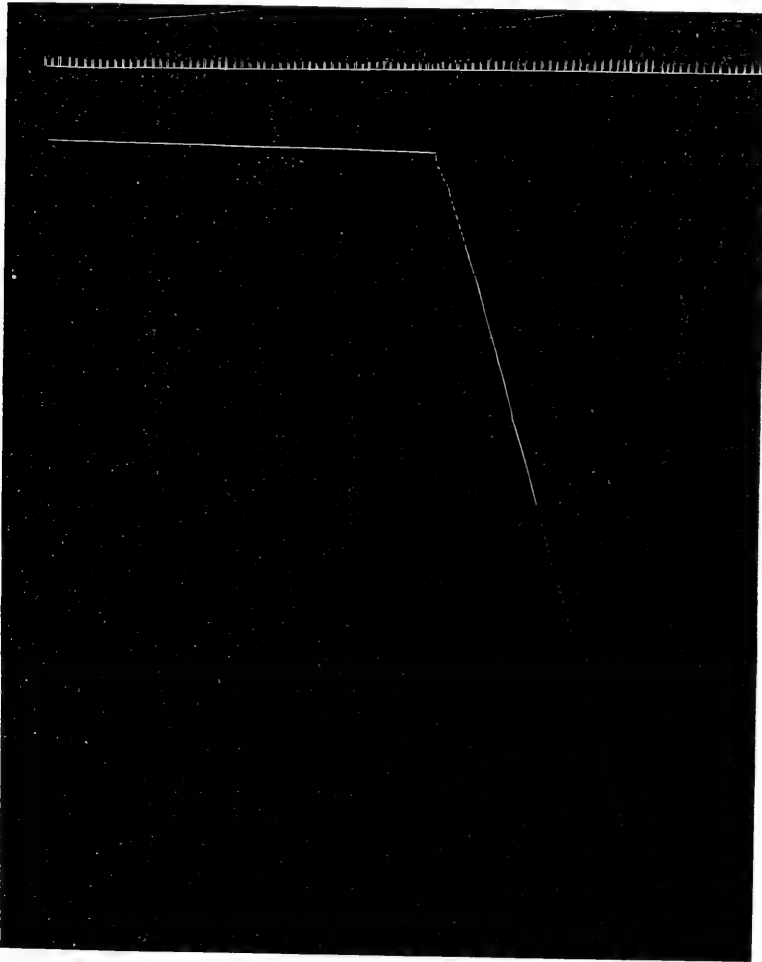


FIG. 2. Record of venous outflow from brain under arterial pressures of 60 mm. and 460 mm. (2d experiment.)

Under 60 mm. Hg. the outflow = 1.74 c.c. per min.

" 460 " " " " = 252.72 " " "

The irregularity in the beginning of the curve showing the outflow under 460 mm. arterial pressure, is owing to a stoppage of the drum, as will be seen by consulting the time-record above it.

brain was still in the unopened cranium, and the physical conditions, which have been supposed to cause a compression of the veins and a temporary or permanent slowing of the blood-flow when the arterial pressure is suddenly raised to supra-normal levels, still prevailed. Indeed, the œdematous condition of the brain should have exaggerated this effect instead of counteracting it. Nevertheless, the records show that in all cases where the arterial pressure was suddenly raised to as much as 400 or 500 mm. of mercury, the outflow of blood from the cerebral veins increased promptly, and there was no indication at all of even a temporary blocking of the flow.

When we consider this fact, together with the results obtained by several authors upon the outflow in living animals in which the arterial pressure was raised by action upon the vaso-motor mechanisms, it seems justifiable to conclude that the blood-flow through the brain is always increased by a rise of arterial pressure, no matter how great or how sudden this rise may be, and that a compression of the veins sufficient to block or temporarily impede the blood-flow as a direct result of a sudden rise in pressure in the cerebral arteries is physically impossible. The author can indorse the conclusion drawn by Reiner and Schnitzler from their experiments that a rise of pressure in the cerebro-spinal liquid due to increase of arterial pressure cannot exceed the simultaneous intravenous pressure.

Obviously the authors who have arrived at an opposite conclusion have erred somewhere in the theoretical premises upon which their argument was based. A satisfactory treatment of all the physical factors involved in the statics and dynamics of the blood-flow through the brain is most difficult, and, perhaps, impossible in the present condition of our knowledge; but it seems to the author that in the theoretical considerations of the subject met with in physiological literature, some factors, which explain in large measure the contradiction between the experimental and theoretical conclusions, have been more or less overlooked. In the first place the view distinctly announced by some authors and tacitly assumed by others that arterial expansion causes a compression of the large venous sinuses of the brain seems to the author to be entirely inadmissible. Grashey calls attention to the anatomical facts that make this view improbable. The venous sinuses are covered by layers of the inextensible dura mater tightly stretched in some cases across bony channels. Any one who examines the



condition of these membranous walls in a fresh skull will be impressed with the opinion stated by Grashey, that the resistance to compression at these places must be so great, as compared with the cerebral veins that open into the sinuses, that any local or general increase of intracranial pressure must affect only the smaller veins. It seems to me, in fact, that the walls of the sinuses are practically incompressible, and that their existing structure is a beneficent adaptation to prevent any interference with the venous outflow arising from arterial expansion, since the venous system is thereby protected from compression at the point where its total cross area would be least, and intravascular pressure at its lowest, and where compression might most seriously affect the venous flow.

I have attempted to demonstrate experimentally the practical incompressibility of the large sinuses, but the method that I have used and which was the only one that seemed to be conclusive, developed so many technical difficulties that I have attained so far only partial success. The method was simple in idea, but somewhat difficult of execution. It consisted in first removing the brain entirely by washing out the tissue through the foramen magnum and a trephine hole in the parietal bone. A flexible rubber catheter was then taken, and upon one end was tied a delicate bag, made usually from the outer coats of the intestine of a frog. The catheter and bag were filled with water from a pressure flask so as to displace all the air, and the catheter was connected by tubing filled with water with a water manometer of barometer tubing. The catheter and bag were then introduced into the transverse sinus through the opening of the superior cerebral vein in the case of the dog, or through the internal jugular when the human skull was used. After the catheter was in position, the pressure in the water manometer connected with it was raised sufficiently to distend the bag and make it lie against the membranous walls of the sinus. Meanwhile the skull had been filled with water, the foramen magnum tightly closed off by a rubber stopper inserted in the sheath of the dura mater, and the trephine hole in the parietal bone connected properly with a mercury manometer and a pressure flask. By means of the latter the pressure within the skull was raised or lowered suddenly to any desired extent, and the effect of this variation in pressure upon the walls of the transverse sinus should have been indicated by the manometer connected with the catheter lying in the sinus.

The experiments of this character made upon human skulls were

not successful owing mainly to the fact that the skulls were obtained from cadavers used in the dissecting room, and the dura mater was so altered as to strip away easily from the bone. In the human skull also there is very great difficulty in getting the catheter through the internal jugular into the transverse sinus owing to the curvature of the channel. In the dog's skull the main difficulty lies in the fact that the transverse sinus throughout most of its extent lies in the bone, only a small portion of it near the torcular having a membranous wall. In other animals, such as the calf and sheep, the transverse sinuses have bony walls throughout their entire course. Another difficulty consists in the fact that the openings of the cerebral veins into the sinuses, particularly into the superior longitudinal sinus, are protected by dura mater so as to remain patent after washing out the brain, and thus make a free channel of communication between the skull cavity and the system of sinuses. This difficulty can be obviated by trephining into the torcular and closing off the sinuses with the exception of the transverse sinus used in the experiment. Still another obstacle is found in the fact that water will filter through the dura mater under high pressures, but the rise of pressure in the sinuses thus produced is gradual and quite different from the sudden rise which would occur if the walls of the sinus were compressed by a quick increase of pressure in the skull cavity.

In one experiment of this kind made upon the dog's skull — and in which subsequent dissection showed that the bag upon the end of the catheter lay properly in the membranous portion of the sinus, it was found that an increase in pressure within the skull up to 500 mm. of mercury caused not the slightest change in the level of the manometer connected with the catheter. This experiment seemed to show quite positively that the membranous walls of the transverse sinus cannot be compressed by intracranial pressures as high as 500 mm. of mercury. Unfortunately corroborative results could not be obtained upon the more favorable human skull owing to lack of appropriate material. But to the author's mind at least the anatomical structure of the sinuses and the physical characteristics of their walls are sufficiently conclusive in showing that normal or supra-normal variations in intracranial pressure cannot affect the calibre of these channels, particularly when we remember that the system of cerebral veins opening into the sinuses offers a relatively very low resistance to compression. As between the cerebral veins and the cerebral capillaries the lower internal pressure prevailing

within the former would seem to ensure that a general rise of intracranial pressure must affect the veins first.

With every increase in arterial pressure there is a tendency to the expansion of the cerebral arteries, but this expansion is only possible when a corresponding amount of liquid is forced out of the brain, the substance of the brain itself being practically incompressible. A clearer understanding of the conditions in the brain has led most authors to believe that room for a sudden expansion of the arteries is made in one of two ways, either by forcing out the cerebro-spinal liquid into the spinal cavity, or by a corresponding compression of the cerebral veins. With regard to the former of these two possibilities exact evidence is lacking of the extent to which it can compensate for arterial expansion, but the facts in our possession would seem to indicate that it plays a minor part. Hill has called attention to the fact that normally the quantity of liquid in the sub-dural and sub-arachnoid spaces is very small, and that under moderate expansion this is expressed into the spinal canal, where room is made for it by expansion of the vertebral ligaments and the possibilities of leakage through the sheaths of the spinal nerves. With a further increase of arterial pressure expansion becomes impossible unless there is a corresponding compression on the venous side, and, as we have seen, this compression should affect first the smaller cerebral veins.

That compression of these veins happens even before the cerebro-spinal liquid is entirely expressed seems to be proved by the occurrence of a pulse in the cerebral veins coincident with the arterial pulse. This venous pulse has been noticed by a number of authors. I have also observed and recorded it a number of times both in the outflow from the superior cerebral veins and from the torcular Herophili. It would seem that this pulse can be accounted for in one of two ways only. It is caused by a wave of pressure transmitted through the capillaries, or it results from a compression of the veins following upon arterial expansion. In the former case there should be a measurable interval between the time of appearance of the pulse in the circle of Willis and in the efferent veins; in the latter case the pulse should be practically simultaneous in the arteries and veins. To determine this point I have made simultaneous records of the pulse in the arteries and veins. The arterial pulse was measured in the circle of Willis by means of a Hürthle spring manometer connected with the head end of the internal carotid; the venous pulse was recorded by a delicate manometer constructed on the principle of

a Hürthle membrane manometer and connected with the torcular Herophili. This connection was made by means of a brass tube screwed into a trephine hole made into the torcular and filled with sodium carbonate solution.

A specimen of the record thus obtained is given in the accompanying illustration.

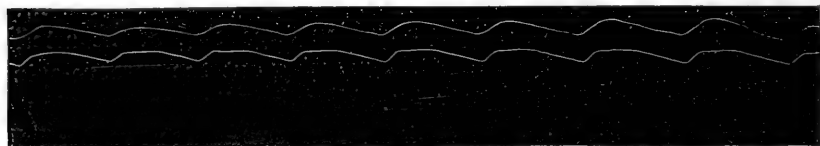


FIG. 3. Simultaneous record of the pulse wave in the torcular and in the circle of Willis (through the internal carotid). The upper curve records the pulse in the torcular, the lower that in the circle of Willis.

It was found that the two pulse waves occurred nearly simultaneously, and that in cases in which any difference in time was detectable the venous pulse slightly preceded the arterial pulse, the maximum difference in extreme cases being as much as 0.01 of a second. This difference is sufficiently accounted for by the stretch of elastic artery (internal carotid) extending from the circle of Willis to the point in the neck where the arterial manometer was inserted. These experiments seem to indicate that the venous pulse is not transmitted through the capillaries, but is due to a positive wave of pressure transmitted through the brain substance by the expanding arteries. This being the case it follows *a fortiori* that a greater general expansion of the arteries of the brain following upon a large rise of arterial pressure should be accompanied by a corresponding compression of the veins. This is the view that Grashey and others have taken, and that has led them to the apparently logical conclusion that a large rise of arterial pressure should result in a compression of the veins sufficient to occlude them temporarily and thus diminish the volume of the blood-flow. The experiments recorded in this paper, however, show that this conclusion is not justifiable. The venous outflow is not impeded even temporarily by a sudden rise of arterial pressure as great as 500 mm. of mercury. It remains for us to explain therefore this apparent discrepancy.

Granting fully that expansion of the arteries causes at once a rise of intracranial pressure sufficient to compress the veins, the fact that the blood-flow is not thereby momentarily impeded may be explained

by two considerations. In the first place in any vascular region the total cross area of the veins exceeds that of the arteries, so that an expansion of the arteries, if accompanied by a resulting symmetrical compression of the veins, would be distributed over a larger cross section and result in a relatively smaller diminution in calibre of the single veins. This consideration in the case of the brain is rendered more important, perhaps, by the fact that the venous system at its termination, namely, in the sinuses, is protected from external compression. The narrowing of the veins occurs, therefore, in the relatively wide area of the small veins, and although the total compression of the veins must equal the total expansion of the arterial stems, the narrowing of the single venous channels is slight, apparently too slight to cause a perceptible obstruction to the blood flow. In the second place the curve of extension of the elastic arterial walls under increasing pressures within follows the general law of extensibility for muscular tissue, showing a gradually decreasing extension for increasing increments of pressure. As the arterial pressure increases, therefore, the expansion of the arterial walls becomes rapidly less, the arteries approach nearer and nearer to the condition of rigid tubes, and the compression of the veins is correspondingly less important. These considerations suffice to explain why an actual occlusion of the veins, such as Grashey assumed, cannot possibly occur, and they explain also, perhaps, why not even a perceptible momentary blocking of the venous flow is obtained under normal and experimental conditions.

As to the permanent effects of arterial expansion upon the resistance to the flow in the veins, several authors have demonstrated quite clearly that even though the compression of the veins were sufficient to bring about a marked resistance to the blood-flow, this effect could only be temporary, inasmuch as the rise of static pressure within the capillaries and veins must always be more than sufficient to again expand the veins. Hill<sup>8</sup> has expressed this view in the following language: "The veins and capillaries, therefore, again become patent, because arterial pressure transmitted directly is obviously greater than arterial pressure minus the tension of the arterial wall transmitted through the brain substance." This author, however, admits that "if the cerebral arteries suddenly expand beyond a certain limit, a process of temporary self-strangulation of these vessels (veins and capillaries) takes place. The circulation itself for a short time stops, and the symptoms of acute cerebral anæmia are

produced." This conclusion, as I understand it, is based entirely upon theoretical grounds, and my experiments show that it is not correct, if it is assumed that the arterial expansion results from a rise of intravascular pressure.

The general conclusion, which seems to me to follow from the experiments given in this paper, is that *a rise of pressure, however great, in the cerebral arteries does not cause directly any impediment to the blood-flow either temporarily or permanently. The circulation in the brain behaves in this respect precisely as it does in the other organs of the body; the greater the arterial pressure the more abundant is the flow of blood, and temporary anæmia cannot be produced in this way.*

If it is found experimentally that a sudden great rise of arterial pressure causes an injurious effect upon the brain, this result cannot be attributed to the direct production of a temporary cerebral anæmia. We can imagine that under these conditions the functional activity of the brain might be interfered with for one of two reasons. It is possible, in the first place, that the resulting rise of intracranial pressure might affect the brain tissues directly; or, secondly, a rapid accumulation of lymph might take place within the brain, which, for a time, would impede the blood-flow, particularly after arterial pressure had again fallen. There is not much probability, however, that this latter effect could occur. Bergmann<sup>9</sup> found that injecting defibrinated blood at high pressures (800–1200 mm. Hg.) into the cerebral vessels of a horse caused no distinct increase in the lymph-flow, and several observers have shown that in other organs arterial hyperæmia alone is not necessarily followed by an increased formation of lymph.

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# THE RECOVERY OF THE HEART FROM FIBRILLARY CONTRACTIONS.

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THE earlier experimenters on the coronary arteries described an apparently fundamental difference between the dog's heart and the rabbit's heart.<sup>1</sup> The heart of the rabbit was found to recover easily from fibrillary contractions brought on by the sudden closure of a large coronary artery, by mechanical injury, or by electrical excitation; but the dog's heart never recovered, either spontaneously, or when attempts at assistance were made by massage of the ventricle, stimulation of the ventricle with single induction shocks, or excitation of the vagus. The improbability of so great a difference between the hearts of two nearly related animals has not been able to outweigh the failure of these efforts to revive the dog's heart, and the idea that the heart of the rabbit and the dog are, in this respect, radically unlike, has obtained a wide acceptance.

It is somewhat strange that the belief in the fatal nature of fibrillary contractions in the dog should have become so fixed in the face of MacWilliam's positive statement that recovery can take place. Almost ten years ago MacWilliam,<sup>2</sup> in his refutation of Kronecker and Schmey's<sup>3</sup> hypothesis that fibrillation was caused by the destruction of a coördinating centre located in the interventricular septum near the junction of the upper and middle thirds, wrote as follows: "There is conclusive evidence that all cases of fibrillar contraction of the ventricle cannot be explained by such an hypothesis — the destruction of a coördinating centre localized as indicated above. The fact that recovery may take place — that the ventricles may resume their coördinated rhythm, controverts the idea of the actual destruction of a centre essential for coördination. Such recovery I have witnessed

<sup>1</sup> The literature of this subject is reviewed by W. T. PORTER, *Journ. of physiol.*, 1894, xv, pp. 121-138; and *Journ. of exper. med.*, 1896, i, pp. 46-70.

<sup>2</sup> MACWILLIAM: *Journ. of physiol.*, 1887, viii, pp. 296-310.

<sup>3</sup> KRONECKER and SCHMEY: *Sitz.-Ber. der Akad. d. Wiss. zu Berlin*, 1884, p. 87.

in several instances in the dog's heart and in a very large number of instances in the hearts of other animals (cat, rabbit, rat, mouse, hedgehog and fowl). Recovery occurs with different degrees of facility in different animals and in different conditions in the same animal. In the dog, recovery occurs with much difficulty and only after the fibrillar contraction has lasted for a considerable space of time; indeed, there very frequently is no recovery apparent — the ventricle may not recommence beating after the incoördinated quivering movement has ceased. At times, however, a number of regular beats are seen after the termination of the fibrillar contraction. . . . In young mammals, foetal or after birth, recovery appears to be the rule; the fibrillar movement is only a temporary condition, and soon gives place to normal beats" (p. 299).

The neglect of MacWilliam's observations by many subsequent writers is perhaps to be explained by their tacit assumption that fibrillary contractions produced by electrical excitation, — the agent used by MacWilliam for their production, — are not the same as those brought on by mechanical injury or by suddenly cutting off the blood-supply, so that recovery from the former does not necessarily prove the possibility of recovery from the latter. The experiments about to be described will show that this assumption has no foundation, and that the heart of the dog recovers from fibrillary contractions produced in any of these ways. It is true that recovery is much more easily obtained in the rabbit than in the dog, but the difference is merely in degree. There is, with regard to recovery from fibrillary contractions, no essential difference between the rabbit's heart and the dog's heart.

In pondering the cause of failure in the many previous attempts to resuscitate the heart, the following considerations became prominent. It is known that the continued coördinated contractions of the mammalian heart are impossible in the absence of a supply of blood to the cardiac muscle. It is also known that fibrillation arising from whatever cause must, by arresting the heart, cut off what has always been considered the only source of blood-supply to the cardiac muscle, namely, the circulation through the coronary arteries. The restoration of the circulation through the coronary arteries would seem, therefore, to be essential to the restoration of continued coördinated contractions; yet restoring the coronary circulation is a means of treatment that has thus far never been tried. The method of attack, then, should be to maintain an artificial circulation of



defibrinated blood through the coronary arteries of the fibrillating heart.<sup>1</sup>

But at this point it was remembered that the rabbit's heart often recovers spontaneously from fibrillation, although the arrest of the ventricle brings the blood-pressure in the aorta so low as to diminish greatly the circulation through the coronary arteries; and Mac-William, as has already been said, saw even the dog heart recover spontaneously. How, it may be asked, can these hearts in which the coronary circulation was so much reduced have recovered without assistance, if the blood supply through the coronary arteries is so important to recovery? An answer to this pertinent question is furnished by two investigations recently made in this Laboratory, the first of which establishes the fact that the quantity of blood necessary for continued coördinated contractions is less than has been supposed, while the second demonstrates that this necessary quantity can be supplied to the ventricular muscle without the aid of the coronary arteries.

In the first<sup>2</sup> of these investigations the intraventricular pressure and the volume of the coronary circulation in the isolated heart of the cat were recorded simultaneously. It was found that the left ventricle would contract vigorously and for many minutes even when the coronary circulation was reduced to very low limits. The small blood-supply on which a good intraventricular pressure and a regular beat can be kept up is surprising. Reference to figure 1, plate 3 of the Paper in question, will show that admirable contractions were secured with about 3 c.c. per minute. In the experiment of March 13, 1896, fair contractions were seen with a coronary circulation of less than one cubic centimetre per minute; but this heart had been isolated a long time when the observation was made. These experiments are evidence that the mammalian heart will work with a blood-supply hitherto supposed to be wholly insufficient for contractions, even against little or no peripheral resistance.

The second<sup>3</sup> investigation shows this fact still more clearly, and shows beside that blood for continued, coördinated contractions can be obtained without the aid of the coronary arteries. In this research the right ventricle of the excised heart of the cat was kept contracting for several hours with no other blood-supply than that

<sup>1</sup> PORTER: Journ. of exper. med., 1896, i, p. 69.

<sup>2</sup> MAGRATH and KENNEDY: Journ. of exper. med., 1897, ii, pp. 13-34.

<sup>3</sup> PRATT: this Journal, 1898, i, p. 86.

obtained from the interior of the right ventricle through the vessels of Thebesius. The ventricle is tied firmly around a glass tube introduced through the pulmonary artery, the ligature passing below the auriculo-ventricular furrow and closing both veins and arteries. Defibrinated cat's blood is then poured into the tube until the ventricle is full and the blood stands in the tube at a height of one or two inches. The right ventricle will now begin to beat, and, if one of the coronary veins on the surface of the heart is incised, a slight but constant stream of blood will flow from the interior of the ventricle through the foramina Thebesii into the coronary veins and out of the opening on the surface of the ventricle. The blood becomes venous in its course through the heart walls. It has never been seen to enter the coronary arteries. Only that ventricle into which the blood is introduced has been observed to beat; the other ventricle remains inactive. Ringer's solution fails to maintain contractions. A ventricle that has fibrillated violently on the excision of the heart will often resume its coördinated contractions when a circulation is established in this manner through the vessels of Thebesius. Thus the nutrition of the mammalian heart may be maintained in a degree sufficient for long-continued, rhythmic contractions, while the coronary arteries are empty.

It should be observed that the state of the ventricle during fibrillation favors this mode of nutrition. Measurements taken in the left ventricle show that the intracardiac pressure rises at this time.<sup>1</sup> The heart becomes greatly distended. Meanwhile, the pressure in the aorta has fallen very low, the ventricle having ceased to beat, and, in consequence, the pressure in the coronary vessels is also very low. Hence the passage of the blood through the vessels of Thebesius into the coronary veins is doubly aided: on the one hand, by the relatively high pressure in the ventricle; on the other, by the diminished resistance in the coronary vessels. In the excised heart of the cat, an intraventricular pressure of two inches of blood will drive a constant stream through the vessels of Thebesius into the coronary veins, as has been already demonstrated, and the intraventricular pressure during fibrillation is probably greater than two inches of blood.

It should be noted further that the peripheral resistance against which the ventricle must work as it recovers from fibrillation is almost nothing, the arterial pressure having been enormously reduced

<sup>1</sup> PORTER: *Journ. of physiol.*, 1894, xv, p. 132.

by the arrest of the ventricle. Thus the heart just recovering from fibrillary contractions and the heart removed from the body are very much alike in respect to the resistance against which the ventricle works. A difference in this resistance cannot, therefore, be urged against the conclusion that the blood-supply which keeps the isolated heart in rhythmic contraction will support the recovering ventricle until it can re-establish the circulation through the coronary arteries.

This same investigation has revealed yet another way in which the heart muscle may be nourished without the aid of the coronary arteries. On June 26, 1897, a cannula was tied into the distal end of the coronary sinus of the freshly extirpated heart of a cat, and filled with defibrinated cat's blood to a height of about 12 cm. Coördinated, regular, and complete contractions then began. The blood flowed from the coronary veins through the fine connecting vessels into the vessels of Thebesius and thence into the cavities of the heart, changing during its passage from arterial to venous. No blood was observed to enter the arteries. The contractions were facilitated by renewing the blood in the cannula from time to time. After twenty-five minutes the heart fell into pronounced fibrillation. Five minutes later the coördinated contractions were resumed, and continued with occasional pauses during almost half an hour. Again interrupted by fibrillation, they again returned, and both fibrillation and recovery were recorded graphically by a writing-lever attached to the apex. After several such attacks and recoveries the heart became exhausted and would beat no more.

The distension of the right auricle during fibrillation is even more favorable to the nutrition of the heart through the coronary sinus than is the distention of the ventricles and auricles to the nutrition through the vessels of Thebesius, for the coronary sinus is large and its valves are weak, falling back before the pressure of a few inches of blood and opening thus an ample way to vascular areas that may not, indeed, be truly capillary, but are none the less composed of vessels thin-walled enough to permit of nutrient osmosis; else why should the extirpated heart beat for hours when fed in this manner, and why should the blood that enters this path arterial red emerge a venous blue?

It is therefore a mistake to suppose that the feeding of the heart muscle is wholly interrupted by the failure of the circulation in the coronary arteries during fibrillation of the ventricle. A significant

supply is still possible. Usually this endocardiac nutrition, if I may term it so, falls below the required amount, and fibrillation continues to the end. In some instances, however, the endocardiac nutrition is sufficient, and the coördinated beat returns. But these unassisted recoveries are very rare, and all that we at present know seems to point to the advantage of liberally supplying the distracted muscle with defibrinated blood.

An artificial circulation of defibrinated blood through the coronary arteries of the fibrillating dog's heart was therefore established. My method of experiment was as follows: The animals were fully anæsthetized with morphia and ether, tracheotomized, the heart and blood vessels exposed by the resection of the first five ribs on the left, and the first three ribs on the right side, and a cannula placed in the left subclavian artery. The innominate artery was then ligated, and running nooses put around the aorta, just distal to the left subclavian artery, and around the ramus descendens and ramus circumflexus of the left coronary artery, near their origin. The cannula in the subclavian artery was connected to a Mariotte's flask of warmed defibrinated sheep or ox blood, placed high enough to give a pressure of 100 mm. Hg. in the aorta. All being in readiness, the nooses around the coronary arteries were drawn tight, until the heart fell into fibrillary contractions. These arteries were then freed, the noose around the aorta drawn tight, the stop-cock between the subclavian artery and the blood flask opened, and a large glass tube hastily tied into the pulmonary artery. The effect of these procedures was to cause the aorta to fill with sheep's blood at a pressure of 100 mm. Hg.; the semilunar valves being thereby tightly closed, and, every outlet but the coronary vessels being also closed, the blood passed through the coronary vessels into the right heart, whence it escaped out of the pulmonary artery into a dish. This blood was then beaten with a glass rod, filtered through glass wool, shaken with air to oxygenate it, and replaced in the Mariotte's flask.

Eight of these experiments were performed,<sup>1</sup> the first on March 7, 1896, and the last on April 23, 1896. The results were interesting, but not decisive. The character of the fibrillation was always modified by the making of the artificial circulation through the walls of the heart. The little contraction waves which cover the actively fibrillating heart were replaced by large undulations. In the experi-

<sup>1</sup> These experiments were done with the assistance of Mr. W. Tileston and Mr. E. DeW. Wales.

ment of March 21, 1896, these large undulatory movements became at times almost regular. On the whole, the impression made was that the ventricles were often on the point of resuming their coördinated contractions, but never altogether did so. Yet it seemed each moment that they would surely beat.

The hope of ultimate success was strengthened by several encouraging observations. The first was the success which attended the effort to restore coördinated contractions to the fibrillating auricle. Thus, in one dog, the heart began to fibrillate at 11.22 A. M.; the defibrinated blood was immediately turned on, and four minutes later the right auricle, which until then had been fibrillating like the rest of the heart, began to beat in an apparently normal fashion. The second encouraging circumstance was that occasionally, though rarely, a part of the ventricle would contract in a perfectly regular manner, while the remainder of the heart was still in hopeless confusion. The part which thus contracted was a small area in the right ventricle near the origin of the pulmonary artery. The third observation was made April 13, 1896. Pronounced fibrillary contractions appeared 215 seconds after the closure of the ramus circumflexus. The defibrinated blood was turned on, and in a short time movements which resembled feeble normal contractions were seen. Fifteen minutes after the beginning of fibrillation, the stop-cock between the blood reservoir and the aorta was turned off, and the supply of blood through the coronary arteries suddenly checked. Violent fibrillary contractions took place when the heart was thus deprived of its blood-supply. On restoring the circulation two minutes afterward, these gave way, and an almost normal beat returned. At the end of five minutes the circulation was again interrupted, and tumultuous fibrillation again appeared, thus showing that the amount of fibrillation was affected by the blood-supply. But notwithstanding these various signs that success was near at hand, no further progress was made at this time, and it was decided to wait until circumstances made it possible to feed the dog's heart with dog's blood.

While the experiments just described were making, other investigations,<sup>1</sup> on the isolated heart of the cat, afforded frequent opportunities to observe the easy recovery which the heart of that animal may make from fibrillation of long duration. When the isolated heart of the cat is fed through the coronary vessels with defibrinated cat's blood, the ventricles usually beat in a fairly normal fashion. Occa-

<sup>1</sup> MAGRATH and KENNEDY: Journ. of exper. med., 1897, ii, p. 14 and p. 30.

sionally, however, strong fibrillation sets in, very fatal to the hopes of the inexperienced operator. But if the experiment is faithfully continued, and the defibrinated blood kept flowing through the coronary vessels, the apparently hopeless ventricle often springs suddenly from its "delirium" into firm, coördinated beats. In one of the experiments published by Magrath and Kennedy, a cat's heart showed marked fibrillary contractions during forty-five minutes and then fell into regular, normal contractions, which continued more than an hour. It was difficult to believe that a disturbance often so transitory in the cat's heart should be always irrevocably fatal in the dog.

The recovery of the dog's ventricle from fibrillary contractions was finally accomplished during the experiments<sup>1</sup> which led to my discovery that any portion of the extirpated dog's heart, even the "ganglion-free apex," will usually resume its coördinated contractions when fed with the dog's own blood at the proper temperature and pressure.

On March 27, 1897, a dog weighing 10 kilogrammes, anæsthetized with morphia and ether, was bled from the left carotid artery, and the blood whipped, strained through glass wool, and diluted with an equal volume of 0.8% normal saline solution. Normal saline of the same strength, made with tap water, and having a temperature of about 36° C., was meanwhile allowed to flow into the right jugular vein. After a short interval the dog was again bled from the carotid artery. The product of these bleedings was mixed and placed in a reservoir at the temperature of the body. The heart was now extirpated, a cannula tied into the ramus descendens of the left coronary artery, the interventricular septum and the auricles completely cut away, and all the ventricle removed except that portion supplied by the descendens itself. The cannula was then connected to the reservoir of warm blood mixture, and the piece of ventricle perfused with the blood at a constant pressure, which to begin with was about 30 mm. Hg., but which was afterwards raised to 90 mm. Hg. The blood entering the cannula was bright arterial red; that emerging by the coronary vein was venous blue. In a few moments the ventricle began to beat with great vigor, shortening about seven millimetres in vertical diameter. An ordinary muscle lever, magnifying eight times, and weighted with 40 grammes, was fastened to a hook thrust through the apex, and recorded curves about 50 mm. in height. The curves showed some irregularity both in force and frequency. The ventricle

<sup>1</sup> PORTER: *Journ. of exper. med.*, 1897, ii, pp. 391-404.

beat more rapidly when surrounded with blood at the temperature of the body than at room temperature, but the character of the contractions remained unchanged. At 1 P. M., after writing curves for one hour, the ventricle was thrown into fibrillary contractions by stroking its surface with the electrodes of a du Bois-Reymond induction coil (tetanic stimulation). Five minutes later good coördinated contractions returned. Forty minutes thereafter the ventricle was thrown a second time into fibrillary contractions, from which it soon recovered. Two and three quarter hours after the ventricle began to beat, the experiment was broken off. The contractions were by this time very feeble, but still unmistakably coördinated.

On March 29, 1897, a similar experiment was made, also on a dog. When the heart was removed from the body and the "apex" excised, the whole heart fell into fibrillary contractions. The right ventricle recovered from these without assistance, giving a few coördinated beats; the apex recovered on being fed with the defibrinated blood from the same dog, through a branch of the descendens. The apex was then thrown into fibrillation by stroking it with the electrodes of a du Bois-Reymond inductorium (hammer in action), but recovered speedily even when very strong currents were used.

The following day the heart of a dog was removed from the chest, and most of the left ventricle and all of the right ventricle and septum, except a fringe near the ramus descendens of the left coronary artery, were cut away. During the cutting of the heart, strong fibrillation appeared. A part of the right ventricle soon recovered spontaneously from this, giving a few fully coördinated beats.

On the afternoon of this day, in another heart, the part of the left ventricle supplied by the ramus descendens was fed through this vessel for nearly an hour without a pause in its ceaseless fibrillation, but finally a brief series of completely coördinated contractions was observed.

April 5, 1897, a still more remarkable recovery was noted. The part of the left ventricle (dog) nearest the apex was removed and fed through its coronary artery. The piece thus extirpated was 10 mm. in length. When good coördinated beats were secured, a powerful induction current was applied, throwing the perfused apex into fibrillation. Recovery took place in a few seconds. The current was at one time so strong as to burn the heart at the electrode points. The apex was now laid on one side in a beaker of blood. After about an hour, the apex was again perfused, and well coördinated,

but feeble contractions secured. Fibrillation was now easily produced with the induction current and continued a long time, but recovery at last took place.

Finally, in two cases, — the only ones in cardiac literature, so far as I am aware, — the dog's right ventricle fibrillated on its removal from the body, and yet, a moment later, unfed and undistended, gave a few coördinated beats. Continued contraction is of course impossible to the ventricle of the dog without a constant supply of nutrient material with which to replenish its rapidly impoverished intramolecular stores.

The experiments thus far related show that the cat's heart recovers readily from fibrillary contractions when the cardiac muscle is fed with the cat's own blood, and that various portions of the dog's heart, — for example, the auricle, the ventricle, and large parts removed from the ventricle, — will recover if fed with dog's blood; and they bring two cases of unassisted recovery, in which the dog's right ventricle, thrown into fibrillation by the extirpation of the heart, gave spontaneously, without perfusion of its coronary vessels, a few regular, coördinated contractions. They do not include any instance in which the whole dog's heart was recovered from fibrillation; but this is not to be wondered at, for the experiments in which the recovery of the whole heart was attempted were made with sheep or ox blood brought from the slaughter-house, and it is well known that such blood is injurious to the heart of the dog. I hesitated some time before attempting the recovery of the whole heart by the perfusion of dog's blood, for it was certain that two dogs would have to be used for each experiment in order to get sufficient blood for the satisfactory perfusion of the entire heart, and it was possible that many animals would be sacrificed to technical difficulties before a successful result could be reached. Moreover, additional experiments seemed unnecessary, for if the auricle and ventricle recover when separated, they should recover when left in their normal connection. This reluctance was strengthened by two preliminary trials, in each of which the whole dog's heart was fed with dog's blood, but without avail, owing to slight imperfections in method. It was therefore resolved to stop the fibrillation, if possible, before attempting to feed the heart muscle, in the hope that coördinated contractions would return if the perfusion was made after every trace of disordered contraction had disappeared. The entire success of this plan is shown by the following experiments.



## *Recovery of the Heart from Fibrillary Contractions.* 81

*Experiment Oct. 25, 1897.* A large dog, anæsthetized with morphia and ether, was bled, perfused with 0.8 per cent sodium chloride solution, bled again, and the blood defibrinated and placed in a pressure-flask at the temperature of the body. The heart of a small dog, anæsthetized with morphia and ether, was then exposed, and the venæ cavæ, the right vena azygos, the aorta, and the innominate and left subclavian arteries ligated. Cannulas were placed in the innominate artery, the pulmonary artery, and the two auricles. The cannula in the innominate artery was connected with the pressure-flask, and supplied the coronary arteries with blood, which, after passing through the heart muscle, escaped from the coronary veins and the vessels of Thebesius and found its way out of the heart by the cannulas in the pulmonary artery and auricles. As soon as the warm defibrinated dog's blood was forced through the coronary vessels, the heart, which had ceased to beat while the preparations were making, began to contract in its regular, normal way. Fibrillation of both auricles and ventricles was now induced by stimulating the ventricles and auricles with a rapidly interrupted current from a du Bois-Reymond inductorium. The auricles soon recovered their coördinated contractions, but the ventricles continued to fibrillate for a little more than an hour. The supply of blood to the heart was then cut off, and iced normal saline solution poured upon the heart until every trace of fibrillation had ceased. The coiled bulb of a surface thermometer placed on the ventricle gave a temperature of  $22^{\circ}\text{C}$ . Blood at  $36^{\circ}\text{C}$ . was again allowed to flow through the coronary arteries. The whole heart then began to beat. The contractions were feeble, but entirely regular and fully coördinated.

*Experiment, November 3, 1897.* The heart of a dog anæsthetized with morphia and ether, was perfused with warm defibrinated dog's blood, as in the preceding experiment. The heart beat very well as soon as the perfusion began. Fibrillation was now brought on by electrical stimulation. The perfusion was then stopped, and iced saline solution poured over the heart until all movement had ceased. On again perfusing the heart with blood at  $36^{\circ}\text{C}$ ., thoroughly good contractions, strong, regular, and perfectly coördinated, began. After this strong and regular beating had been watched for some time, fibrillation was again induced, and the heart cooled down, as before. But perfusion was this time recommenced before the last tremor had ceased. The result of this premature action was the return of fibrillary contractions in full force. Twice again the heart was cooled and perfused before fibrillation had wholly ceased, with the same result. Then still another cold bath was given, and this time perfusion was not begun until the ventricle lay quiet. The heart now beat in normal fashion for a long time, the contractions being very forcible and completely coördinated.

Thus the whole dog's heart can be recovered from fibrillary contractions by cooling the ventricles until all trace of fibrillation has disappeared, and then bringing the heart back to the normal temperature by circulating warmed defibrinated blood through the coronary vessels. Doubtless the whole heart of the dog, like the auricles and ventricles, and like the heart of the cat and the rabbit, can also be recovered by persistent feeding with defibrinated blood at normal temperature and pressure, and, in very rare cases, by endocardiac nutrition through the vessels of Thebesius and the coronary veins, but further experiments would only confirm the statements contained in these pages. The thesis with which this Paper began has been sufficiently demonstrated. In respect of recovery from fibrillary contractions, there is no essential difference between the hearts of the rabbit, the cat, and the dog.

## NOTES ON THE ELIMINATION OF STRONTIUM.

BY HORATIO C. WOOD, JR., M. D.

[*From the Chemical Laboratory of the University of Pennsylvania.*]

IN H. C. Wood's Therapeutics it is affirmed that the absorption of the strontium salts and their elimination *appears* to be rapid, but the only exact chemical studies of the matter which I have been able to find are those of Laborde, *Compte rendu de la Société de biologie*, Paris, 9 s., 1890, vol. ii, pp. 453 and 708, and 1891, vol. iii, p. 562. In one of Laborde's experiments an amount of the strontium sulphate sufficient to represent 117 gm. of the metallic strontium was administered to a dog by the mouth in the course of 81 days, and the metal was found in the urine, fæces, bones, and liver. In a second experiment, the tartrate having been given in such quantity as to represent 453 gm. of the metallic strontium, that substance was again detected in the urine, bones, and liver, but was especially abundant in the fæces. In a third experiment, strontium phosphate representing 265 gm. of the metal was given in 111 days; in the urine and liver only unweighable traces were found, — from the bones was separated 0.63 gm. of the metal. Except in regard to the bones in the last experiment mentioned, Dr. Laborde does not report any amount of weighed metal obtained.

These experiments of Laborde do not prove that strontium is rapidly absorbed or eliminated, but only that it is absorbed to some extent and slowly rather than rapidly thrown off. For the purpose of obtaining more exact data I have made two experiments. The method of separating the strontium, for which I am indebted to Professor John Marshall, is as follows: —

"The urine and fæces were separately evaporated to dryness, and the residue incinerated until the organic matter was completely destroyed. The residue was warmed with nitric acid, the solution diluted with water, filtered, the filtrate nearly neutralized with ammonium hydroxide, and ammonium carbonate added in slight excess. The liquid was boiled, and the precipitate collected on a filter paper and thoroughly washed. The precipitate was then dissolved in just sufficient acetic acid to bring it into solution, and the solution then diluted with water. To remove phosphoric acid ferric

chloride was added and the solution nearly neutralized with sodium carbonate. The liquid was boiled, and filtered while hot to remove the triferric phosphate and basic ferric acetate which had separated. The filtrate was evaporated to dryness on a water-bath, and the residue repeatedly treated with nitric acid, evaporating the solution to dryness after each addition of nitric acid to convert the calcium and strontium present into nitrates. The dry residue was pulverized and treated with a mixture of strong alcohol and ether to separate calcium nitrate from strontium nitrate. The residue remaining after treatment with strong alcohol and ether was tested by means of the flame test for strontium, and wherever the quantity of residue remaining indicated a weighable quantity of strontium the residue was dissolved in a small quantity of water and the strontium precipitated by the addition of dilute sulphuric acid. In such cases the liquid was permitted to stand 12 hours, when the precipitate of strontium sulphate was collected on a filter paper, washed with a mixture of alcohol and ether, dried, incinerated, and weighed."

The first experiment was upon myself. In it I took by the mouth 3 grammes of strontium lactate ( $\text{Sr}(\text{C}_3\text{H}_5\text{O}_3)_2$ ) representing 1.89 grammes of metallic strontium. The urine and fæces were collected separately for forty-eight hours. The result may be presented conveniently in a tabular form as follows: —

*First day.*

9 a. m.	Ingestion of 3 gm. strontium lactate.
10 a. m.	Urine contains a trace of Sr.
2 p. m.	" " " "
6 p. m.	" " " "

*Second day.*

9 a. m.	Fæces contain 0.0223 gm. metallic Sr.
2 p. m.	No strontium in urine.
10 p. m.	" " "

*Third day.*

9 a. m.	Fæces contain 0.1503 gm. metallic Sr.
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Unfortunately the fæces were not saved after the third day, but it will be noted that during the forty-eight hours after the ingestion of an amount of strontium lactate representing 1.89 grammes of the strontium, 0.1706 gramme of the strontium was recovered from the fæces, equalling about 10 per cent of the amount ingested, whilst only a trace was found in the urine. This result is capable of several explanations; it is possible that the strontium is not absorbed and very slowly passes out from the alimentary canal, owing to its weight

causing it to cling to the mucous membranes; or it is possible that being more largely absorbed than is apparent it is eliminated with great slowness. The latter conclusion, however, is highly improbable in the face of the fact that the strontium disappeared from the urine within twenty-four hours. If absorption and retention were the case, certainly there should have been as much strontium in the urine on the second as on the first day. It is of course possible that the discharge of strontium with the fæces may have depended upon an elimination of absorbed strontium by the intestinal mucous membrane.

To determine whether such elimination occurs to any extent a second experiment was made. In this a small dog was given a hypodermic injection of 3 grammes of strontium lactate, dissolved in water. The urine and fæces were separately collected for 72 hours, and carefully examined by the process previously given, but no strontium was detected.

The experiment just recorded shows that strontium given hypodermically, if absorbed at all, is only eliminated with the greatest slowness; and demonstrates that the strontium which was passed from the bowel in the first experiment had not been eliminated from the bowel but had remained unabsorbed. As suggested by Professor Marshall, it is probable that when the strontium salt is taken by the mouth absorption takes place to some extent in the stomach, but that that portion of the salt which escapes into the intestines is converted into an insoluble phosphate. It is also probable that the alkaline juice of the tissues largely breaks up the strontium salt, so that absorption from the cellular tissue is a very slow process. It is a further plausible conclusion that the strontium salts taken into the intestines, like the bismuth salts, are so very slowly absorbed that they exert a persistent local influence; an experimental conclusion which is in accord with the clinical fact which has been insisted upon especially by Professor H. C. Wood, that the strontium salts have a marked influence upon the digestive processes in the intestinal canal.

# THE NUTRITION OF THE HEART THROUGH THE VESSELS OF THEBESIIUS AND THE CORONARY VEINS.<sup>1</sup>

BY F. H. PRATT.

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FEW beliefs of the present day are more firmly intrenched than that of the total dependence of the mammalian heart upon the coronary arteries. We are taught that without the blood brought by these vessels the long-continued, rhythmic contractions of the cardiac muscle are impossible. Such is the foundation on which rest in large part the prevailing ideas of infarction in the heart, with all the train of evil consequences believed to follow the embolism and thrombosis of the coronary arteries. It is my purpose to show that this doctrine is not absolute. The long-contracting, rhythmic heart is not wholly dependent upon the coronary arteries for its food supply; on the contrary, the heart will beat for hours while its arteries are empty. There are two ways in which the heart muscle may thus be nourished: the first, through the vessels of Thebesius, which open from the ventricles and auricles into a system of fine branches communicating with the cardiac capillaries; the second, through the coronary veins, which may convey a backward flow of blood from the auricle into the tissues of the heart.

So far as I have been able to determine, no experimental physiological work has ever before been done on the vessels of Thebesius; all opinion regarding their functional importance has rested upon the assumption that they serve only as veins, conveying a part of the venous blood from the coronary capillaries through the foramina Thebesii into the cavities of the heart. The question, too, of the nutritive significance of regurgitation from the auricle into the coronary veins has not apparently at any time been the object of investigation.

<sup>1</sup> The first account of these experiments was given to the American Physiological Society in May, 1897 (see *Science*, June 11, 1897). The subject was presented also to the Boston Society of Medical Sciences, June 1, 1897, and to the British Association for the Advancement of Science, Toronto, August, 1897

The following pages will present an historical sketch of the anatomy of the vessels of Thebesius, and a record of my own anatomical studies of these vessels and of the coronary veins; a detailed account of my experiments on the nutrition of the heart through the vessels of Thebesius and the coronary veins will follow; and, finally, I shall endeavor to show that these forms of nutrition afford a reasonable explanation of the recovery of the heart from fibrillary contractions and from acute distentions leading to arrest, and of the extraordinary fact that hearts may work for years in spite of the almost complete obstruction of their arteries by advanced arterio-sclerosis.

## I. THE ANATOMY OF THE VESSELS OF THEBESIIUS AND THE CORONARY VEINS.

**The Vessels of Thebesius.** — The discovery of the foramina Thebesii is credited to Vieussens;<sup>1</sup> but we owe the first accurate description of them to Thebesius,<sup>2</sup> whose valuable work, *De circulo sanguinis in corde*, was published by A. Elzevier in 1708. Nearly fifty years later we find Haller<sup>3</sup> describing "still more, and much smaller, veins in the heart, whose little trunks, being very short, cannot easily be traced by dissection; and these open themselves by an infinite number of oblique small mouths, through all the numerous sinuosities observable on the surface of the right and left ventricle. These are demonstrated by injections of water, wind, or mercury; pushed into the coronary arteries, after you have first tied their corresponding or accompanying coronary veins; or even into the great coronary veins, after you have first intercepted the openings of their largest trunks. For, in either of these cases, there are drops of the tinctured water, bubbles of air, spherules of mercury, rushing out through the whole extended surfaces of both ventricles of the heart; and this, without any violence that can be supposed sufficient to break the vessels. But the passage from the arteries into the cavities of the left side is more difficult." Notwithstanding these various statements the existence of the vessels remained for many years a subject of dispute. Thus Abernethy<sup>4</sup> early in the present century, commenting

<sup>1</sup> VIEUSSENS: *Nouvelles découvertes sur le cœur*; 1706; quoted by HALLER: *Elementa Physiolog.* Lausanne, 1757, lib. iv, p. 380.

<sup>2</sup> THEBESIIUS: *De circulo sanguinis in corde*. Leiden, 1708.

<sup>3</sup> HALLER: *First lines of physiology*; English translation. Edinburgh, 1786, vol. i, p. 75.

<sup>4</sup> ABERNETHY: *Philosophical Transactions*. London, 1798, p. 103.

on the perplexities of his predecessors, pointed out that even Haller, Senac, and Zinn were sometimes unable to discover the foramina, and were led to suspect that their apparently successful injections really ruptured the vessels and forced a way through false passages into the cavities of the heart. Abernethy himself made many injections, filling the arteries and veins with wax of different colors. He convinced himself that the foramina actually existed; and that they belonged moreover to both the arteries and the veins, because "the injection which was employed was too coarse to pass from one set of vessels to the other, and yet the different coloured injections passed into the cavities of the heart unmixed." His conclusion that some of the foramina communicate with coronary arteries is, I think, hardly to be accepted in the light of other observations. It is probable that in his experiments also extravasations took place from the arteries into the ventricles.

Bochdalek,<sup>1</sup> in 1868, published the results of observations on the foramina Thebesii of the auricles. He found in both auricles the mouths of small vessels. Many of these openings presented the appearance of blind depressions, since they were often covered with single valves in such a way as to resist investigation with the blowpipe. When he succeeded in introducing an injection-mass or a blast of air into a foramen, superficial, ramifying vessels were demonstrated, the injection of which often showed a connection with other foramina in the same auricle, or even in that of the other side. The foramina varied considerably both in number and in size; some were round and small; others were slit-like, resembling the mouths of the ureters; still others were large, round depressions, with smaller openings at the bottom. As a result of his observations, Bochdalek concludes "that the greater number of the small openings on the inner surface of the right as well as the left auricle, which from early times have borne the name of foramina Thebesii, represent the mouths of little veins that, often uniting into larger vessels, course with many branches through the auricular walls."

In 1880 appeared the report of Langer's<sup>2</sup> research on the foramina Thebesii of the human heart. With the aid of the blowpipe, and by means of a watery injection mass colored with Berlin blue, he demonstrated these foramina in all the cavities of the heart. He

<sup>1</sup> BOCHDALEK: *Arch. f. Anat., Physiol. u. wiss. Med.*, Leipzig, 1868, p. 314.

<sup>2</sup> LANGER: *Sitzb. der k. Akad. der Wissensch. zu Wien*, 1880, Bd. lxxxii, 3. Abth., p. 25



succeeded in injecting the vessels of Thebesius not only from the coronary vessels, but from the endocardial surfaces as well. Bochdalek's observations regarding the presence in both auricles of foramina Thebesii were thus confirmed, and the fact of a communication between the coronary vessels and each of the four cavities of the heart was thoroughly established. The foramina which Langer found on the endocardial surfaces of both ventricles were similar to those in the auricles, but much smaller. They were most conspicuous on the papillary muscles and in the neighborhood of the great vessels, being less easily seen in the region of the apex, where they were obscured by the trabecular network. Injections from the endocardial surfaces showed fine, ramifying vessels connected with the foramina, running either at right angles to the surface, or obliquely. From the fact that thick injection masses would not pass from the coronary veins into the ventricles, and that even watery masses were slow in appearing there, Langer concluded that the foramina Thebesii of the ventricles were not, as occasionally happens in the auricles, in direct communication with the coronary veins, but that they had to do with separate capillary areas. In no case did he observe valves in connection with the foramina Thebesii of the ventricles.

Gad<sup>1</sup> has recorded some confirmatory observations on the vessels of Thebesius in the ox. In the method which he describes for demonstrating the action of the valves of the left heart, wherein water under pressure is made to fill the ventricle and aorta, he noticed that water flowed into the right heart from the foramina Thebesii. On illuminating the interior of the left ventricle he was enabled to see fine, blood-stained streams, issuing from the endocardial wall into the clear water with which the cavity was filled.

Finally, Magrath and Kennedy,<sup>2</sup> working with artificial circulations of defibrinated blood on the isolated heart of the cat, observed that a small portion of the coronary blood found its way into the left ventricle. The only possible source of access, other than from the vessels of Thebesius, was leakage past the aortic valves. This leakage, as shown by a manometer record of the aortic pressure, did not occur.

Notwithstanding these painstaking observations, the vessels of Thebesius still occupy a very obscure position in anatomical literature. Foramina Thebesii are referred to as constant in the right

<sup>1</sup> GAD: Arch. f. Physiol., 1886, p. 380.

<sup>2</sup> MAGRATH and KENNEDY: Journ. of Exp. Med., 1897, ii, p. 13.

auricle, forming in part the mouths of small veins. Their occurrence in the left auricle is occasionally mentioned. But the fact that vessels of Thebesius open into all the chambers of the heart—ventricles as well as auricles—is hardly recognized.

The anatomical methods employed in the present study have been three in number; the injection of the vessels with air by means of the blowpipe, their injection with liquids, and the making of corrosion preparations. My experiments have in the main confirmed the results of other observers.

The initial step was to demonstrate independently the fact of a circulation in the vessels of Thebesius. By the injection of water, normal saline solution, or defibrinated blood, at a constant pressure, I established an artificial circulation through the coronary vessels of the fresh, often the still living, extirpated hearts of the rabbit and the dog. The cannula was tied directly into a coronary artery or one of its branches, so that access of liquid into the heart-cavities, except through the endocardial foramina, was rendered impossible. Yet liquid constantly found its way through the vessels of Thebesius. Thus I was enabled to verify the results of Magrath and Kennedy touching these vessels. It is essential to success that the heart be used before rigor has set in, and that the coronary system be thoroughly washed out by the injection of water or normal saline solution into the aorta. The following experiment will make the procedure clear.

*February 8, 1897.* A dog was anæsthetized with morphia and ether, and the heart excised after contractions had ceased. The coronary system was rinsed with normal saline solution introduced from the aorta. The systemic and pulmonary veins and the right coronary artery were ligated. A cannula was passed into the left coronary orifice from the aorta, and tied in the descending branch of the left coronary artery. The walls of the aorta were drawn close to the shank of the cannula by a ligature. Two outflow tubes were now arranged; one in the right ventricle through the pulmonary artery, the other in the left ventricle through the left auricular appendix. Normal saline solution colored with defibrinated blood was passed through the cannula under a constant pressure of 85 mm. Hg. and collected from each outflow tube separately. The amounts were then measured and compared. The quantity of the solution collected during five minutes was 400 cc. from the right ventricle, and 4 cc. from the left.

After thus assuring myself that a genuine communication exists between the coronary vessels and the left heart, it remained to in-

quire particularly into the nature of this communication. The heart of the ox was found best suited to the purpose. An examination of the endocardial surfaces of the ox heart reveals irregularly placed depressions, usually sharply outlined, varying widely in size, shape, and distribution. They are regularly larger in the auricles than in the ventricles. In the right auricle they may be provided with thin, single valves, especially about the origin of the great veins. In the left auricle they are usually fewer in number, and, so far as my observations have gone, unprovided with valves. Foramina Thebesii are never absent from the ventricles. In the right ventricle, which is especially well provided with them, the larger number are seen upon the septal wall. It is often much more difficult to find them in the left ventricle, although a diligent search is never without reward. Here, in agreement with Langer's statement, they often appear at or near the bases of the papillary muscles. They may present themselves in either ventricle almost anywhere on the endocardial surface. Structures, accessory to these ventricular foramina, which might in any way serve the office of valves, I have not seen; the edge of the foramen is usually sharply defined and may frequently exist as a partial, shelf-like covering, giving the impression, perhaps, of an attempt at a membranous valve; but it is seldom more than this.

Experience soon teaches one to distinguish, often at a glance, between the foramina Thebesii and merely blind depressions in the endocardium. On the injection of the vessels of Thebesius with air by means of the blowpipe applied to the foramina, characteristic, fine, sub-endocardial ramifications, which very frequently conduct the air into other Thebesian systems or even into the great coronary veins, will seldom fail to appear. Connection with the coronary veins may be further established by injection from the veins themselves. The following observations will illustrate these points.

*April 7, 1897.* — The right ventricle of a fresh ox heart was opened from the tricuspid valve to the apex. A large number of foramina were seen on the septal endocardial wall. The inflation with the blowpipe of vessels leading from these was in most instances followed by the appearance of air-bubbles at the mouth of the coronary sinus. On tracing the source of the bubbling, the air was observed to come from the mouth of a coronary vein at the extreme end of the sinus. This vein was now opened, and a cannula tied into the distal end. Defibrinated blood, forced through the cannula by blowing into the end of an attached rubber tube, was plainly seen to emerge from the foramina.

The ease with which injections of air and blood could be made to demonstrate the connection between the vessels of Thebesius and the coronary veins caused me to doubt the opinion expressed by Langer, that the foramina Thebesii in the ventricles communicate with the veins by capillaries alone. To settle this point I injected the coronary veins of the ox with starch and celloidin masses, both too thick to pass the capillaries, and found that even these emerged from the foramina Thebesii of the right ventricle. So intimate a connection, however, between the coronary veins and the vessels entering the left ventricle I have not yet been able to demonstrate.

By means of a very successful corrosion preparation, made by injecting the veins of an ox heart with celloidin, I was able to trace the communication. In this preparation the position of some of the foramina Thebesii was marked by small discs of the hardened mass, formed by the oozing out of the celloidin upon the endocardium. These foramina were shown to be connected with the smaller coronary veins by fine branches. The still finer ramifications which, as Langer has demonstrated, lead from the foramina and branch directly into capillaries were here uninjected; they would appear only when injected from the foramina themselves.

Although I have made attempts, I have been unable to discover anything more free than a capillary connection between the vessels of Thebesius and the coronary arteries. Injections of starch or celloidin fail to pass from the arteries into the cavities of the heart.

Bochdalek's observation, relative to a communication between the auricles through vessels of Thebesius, I have verified on the heart of the dog. Blowpipe injection of a foramen in the left auricle caused an exit of air from a similar foramen in the right auricle, and this without any discernible inflation of a coronary vein.

I have no reason to believe that any of my results have been due to the rupture of vessels and the consequent extravasation of the circulation-fluid or injection-mass. Care has been taken throughout to avoid high pressures, and this, together with the very important consideration that my material has all been fresh, would serve to render errors resulting from this cause very improbable.

The vessels of Thebesius, therefore, open from the ventricles and auricles into a system of fine branches that communicate with the coronary arteries and veins by means of capillaries, and with the veins — but not with the arteries — by passages of somewhat larger size.

**The Coronary Veins.** — Whenever in my experiments a circulation-fluid has been permitted to gain access to the coronary sinus from the right heart, the anatomical possibility of a back-flow into the veins has been demonstrated. I have taken pains to supplement these observations with dissections of the coronary veins in the cat, dog, and ox. Everything has pointed to the fact that such valves as the veins of the heart possess are very inefficient. A single membranous fold is usually found at the entrance of a vein into the sinus; seldom does it prevent the entrance of air or liquids. Valves at the confluence of venous branches I have found either wanting or very poorly developed. The Thebesian valve at the mouth of the coronary sinus is totally insufficient to prevent regurgitation.

## II. THE NUTRITION OF THE HEART THROUGH THE VESSELS OF THEBESIUS.

The experiments which I shall now describe were suggested by the possibility of an arterial function on the part of the vessels of Thebesius. They have served to prove not only that these vessels may carry nutriment to the heart-muscle, but that in the absence of all other forms of cardiac nutrition — with the blood-supply through the coronary arteries absolutely cut off — the mammalian ventricle may be maintained, under proper conditions, in rhythmic contractions lasting several hours.

Nearly all of the experiments have been made upon the heart of the cat. The animal is anæsthetized with ether, tracheotomized, and bled from a cannula tied into a carotid artery. The blood is defibrinated and filtered through glass wool. On cessation of the bleeding the ventral chest wall is at once removed by cutting through the costal cartilages, and the heart quickly severed from its connections. If care has been taken with the etherizing, respiration should have persisted up to the time of opening the thorax, and the excised heart should be beating with perfect regularity; fibrillary contractions need not, however, interfere with success. The heart is rinsed in warm normal saline solution. A cannula about the size of the aorta is now introduced into the right ventricle through either the auricle or the pulmonary artery, and secured by a ligature passed tightly around the base of the heart in a line parallel to the auriculo-ventricular groove and below the coronary sinus. Thus a fluid introduced into the ventricle through the cannula can find no outflow except through the vessels of Thebesius. The cannula is now supported in an

upright position, with the heart suspended from the lower end, and the defibrinated blood poured in until the ventricle is full and the blood rises in the tube to a height of several inches. Figure 1 will make clear the relation of the cannula to the ventricular cavity. Figure 2 represents a form of perfusion cannula and a heating apparatus which have been found very convenient for supplying fresh blood constantly to the ventricle, and for maintaining the heart at body-temperature.



FIGURE 1. Diagram to illustrate the mode of securing the cannula in the ventricular cavity for nutrition through the vessels of Thebesius.

As a result of the above method — often within a minute after the introduction of blood — strongly marked, regular, coördinated contractions of the ventricle are observed. With a periodic supply of fresh blood, and with favorable temperature and moisture, this activity may continue several hours. The following are typical experiments.

*April 1, 1897.* An etherized cat was tracheotomized and bled from the left carotid artery. The heart was excised while beating, and rinsed in saline solution. A cannula was passed into the right ventricle through the right auricular appendix, and secured by a ligature tied tightly about the base of the heart. The heart had fibrillated immediately after excision, and shortly had ceased all movement. The cannula and the ventricle were now filled with defibrinated blood diluted with one part saline solution. The ventricle straightway began to beat with great regularity. On suspension of the heart in saline solution of normal temperature the beat was increased in frequency. The blood, rising in the tube about 15 mm. with each beat, became gradually venous in color. The frequency of contraction then diminished, but was restored by replacing the venous with arterial blood. No diminution in the amount of the blood was noticeable, and no blood could be seen to issue from the heart. The coronary arteries were empty; the veins were filled over the entire surface. Tracings were taken by attaching a rubber tube connected with a Marey tambour to the top of the cannula (Fig. 3). The resistance thus occasioned gradually lessened the frequency of the heart-beat, but the heart immediately recovered its former rate on removal of the tambour-tube.

The heart began beating at 4.55 P. M. At 6.20 it was still beating slowly. The blood had been renewed several times.

An experiment of April 2, 1897, showed that a true circulation may take place between the vessels of Thebesius and the coronary veins. Here the conditions were the same as in the experiment just

recorded, except that two of the veins were incised. A small but steady stream of venous blood issued from them.

On April 3, under the same conditions, the descending branch of the left coronary artery was opened. No flow of blood occurred from the artery, although there was a free escape from an incision in an accompanying vein.

The following experiment shows that the above form of nutrition is not confined to the right ventricle.

*April 7, 1897.* The left ventricle of a cat was nourished with blood by the same method, except that the trunks of both coronary arteries were ligated, and the ligature about the ventricles omitted. The supply cannula was tied into the ventricle through the aorta. On the introduction of blood the left ventricle alone began to beat strongly and regularly. The height of the blood column was about 80 mm. A small but continuous flow of blood occurred from a cut vein. The beating continued for over an hour, becoming weak at last, but remaining regular. The blood found its way in part into the right ventricle, coming of necessity through the walls. The blood had been changed frequently by perfusion, and had invariably become venous in color. The temperature was kept at 30–36° C.

In considering these experiments the suspicion very naturally arises that the prolonged contractility of the filled ventricle may be due, not to the nutritive property of the blood, but to mere mechanical stimulation brought about by distention. Evidence that such distention is in itself a powerful stimulus to contraction has recently been furnished in this Labo-

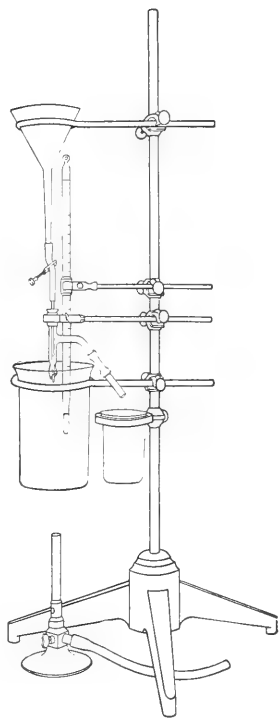


FIGURE 2. Perfusion apparatus for feeding from the interior of the heart-cavities. Blood placed in the funnel is allowed to pass very slowly through the fine glass tube into the ventricle, which is tied upon the cannula as illustrated in Figure 1. The supply is regulated by the compression clamp. Each beat of the ventricle forces the blood back through the space between the fine inner tube and the walls of the cannula, out through the side branch, and thence into the small receiving beaker. The large beaker is filled with saline solution kept at normal temperature.

ratory.<sup>1</sup> But mechanical stimulus is inadequate to explain the phenomenon; for when Ringer's solution of sodium, potassium, and calcium chlorides<sup>2</sup> is used alternately with blood under constant conditions, the solution fails to sustain contractions, while the blood succeeds. The following experiment is an example.

*April 10, 1897.* A cat's heart was prepared in the usual way, with the cannula in the right ventricle. Ringer's solution at 35° C. was now introduced instead of blood. A slow beating of the right ventricle began: during the first five minutes, 30 beats; during the second five minutes, 6 beats. The contractions were at first strong, but gradually became irregular in force, and spasmodic; they finally stopped. During a third five minutes there were no beats. The solution was now replaced by blood. The beating returned: during the first five minutes, 44 beats; during the second five minutes, 174 beats (60 during the last minute). The contractions were strong and regular throughout fifteen minutes; and later, when the blood was removed and the ventricle washed out, were still seen.

An immediate repetition of the above procedure was followed with similar results; Ringer's solution failed to sustain contractions, while blood caused long-continued beating.

There can remain, then, no doubt of the genuinely nutritive character of the phenomena observed under this method; for the blood enters the ventricle as arterial blood and emerges into the veins of a dark venous color, while rhythmic beats are maintained for a much longer period than can be accounted for on other grounds. Since all other possible channels have been cut off, the veins, which fill with blood, can communicate with the ventricle only by means of the vessels of Thebesius. The coronary arteries can take no part in the circulation, since they are found empty.

One of the conspicuous features in the experiments of Magrath and Kennedy was the fact that an exceedingly small blood-supply was sufficient to maintain the unloaded heart of the cat in regular contractions. These authors published a graphic record of excellent beats which were sustained by a coronary circulation of about 3.3 c.c. per minute, and report a case of fair contractions observed under a circulation of less than 2 c.c. per minute; the customary volume

<sup>1</sup> HYDE (I. H.): Proceedings of the American Physiological Society, Science, Jan. 22, 1897. The paper will be published in full in this Journal, 1898, vol. i.

<sup>2</sup> Modified for mammalian tissues: NaCl, 0.9%; CaCl<sub>2</sub>, 0.02%; KCl, 0.01%; water distilled in glass. The formula was kindly furnished by Mr. F. S. Locke.



employed ranged from 3.5 c.c. to 13 c.c. per minute. My own results serve to emphasize this fact still further. In supplying the heart with blood through the vessels of Thebesius the circulation was at all times very small—in many cases, even, hardly measurable. In the experiment of April 1, there can have been scarcely more than a mere oozing of blood in and out between the coronary veins and the ventricular cavity through the vessels of Thebesius.

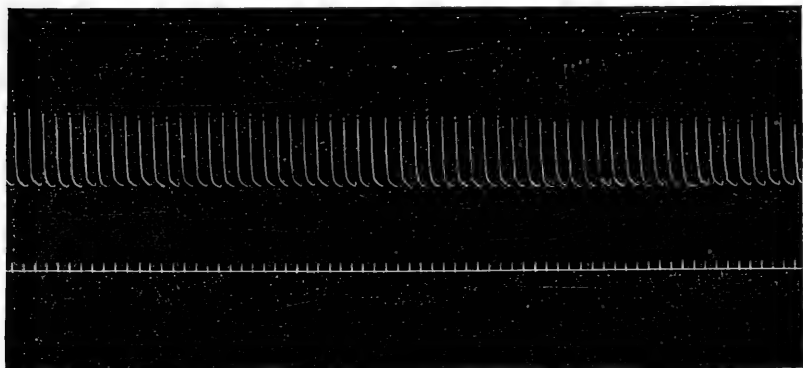


FIGURE 3. From a cat's heart fed from the interior of the right ventricle, April 1, 1897. Recorded one hour after removal of the heart from the body. The upper curve was drawn by means of a Marey tambour connected with the cannula in the right ventricle. The lower curve gives the time in seconds.

The discussion of the nutrition of the heart through the vessels of Thebesius leads now to the consideration of a somewhat striking analogy. It is known that the heart of the frog receives almost its entire nutriment through the branching passages that carry the blood from the interior of the heart nearly to the pericardial surface. My experiments have shown that the heart of the cat may be nourished in much the same way; there is, indeed, a marked resemblance in both method and results between my experiments and many which have heretofore been performed on the heart of the frog. The simplicity of this method of nutrition, as well as its value in bringing the mammalian and batrachian hearts in a physiological sense closer together, seems to assure its further usefulness in experimental work.

### III. THE NUTRITION OF THE HEART THROUGH THE CORONARY VEINS.

The possibility of a nutrition through a back-flow from the auricle into the coronary veins was first suggested by an experiment per-

formed April 5, in which the right ventricle was prepared in the usual way, except that the ligature encircling the heart was omitted. The cannula was tied into the pulmonary artery. Blood was thus allowed to enter the right auricle through insufficiency of the tricuspid valve. The heart was sustained in rhythmic contractions for eight hours,—a period considerably in excess of that observed in nutrition through the vessels of Thebesius alone. It was inferred that

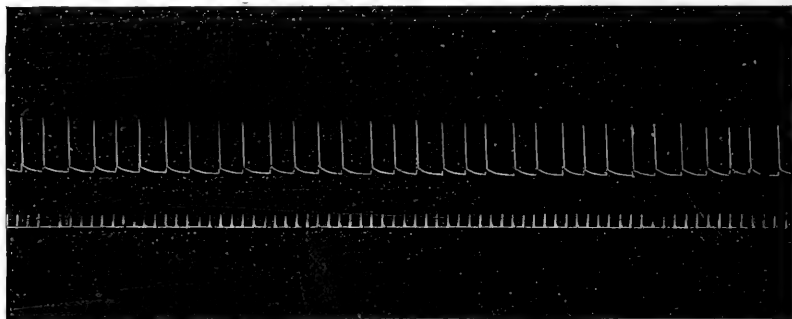


FIGURE 4. From a cat's heart fed from the right ventricle and auricle, April 5, 1897. Recorded six hours after the removal from the body and the beginning of contractions. The upper curve was drawn by means of a Marey tambour connected with the cannula in the pulmonary artery. The lower curve gives the time in seconds.

blood had gained access from the auricle to the coronary veins, and had thus aided materially in the nutrition. The question was therefore submitted to experiment, as follows:—

*June 26, 1897.* The coronary sinus of a freshly extirpated cat's heart was opened at its middle, and a cannula tied into the distal end. This cannula was supported vertically, and filled to a height of 12 cm. with defibrinated arterial blood. A few minutes after the introduction of the blood both ventricles began to beat. The contractions were coördinated, regular, and complete, and took place at intermittent periods for an hour and a half; they were facilitated by frequent renewals of the blood. The amount of blood contained in the cannula was never more than 4 c.c. During the experiment numerous recoveries from fibrillary contractions were observed, and records secured during the process of recovery. (Fig. 5.)

The above experiment makes evident the fact that under very low blood-pressure the unloaded heart may be made to beat for a long time by feeding it through the coronary veins alone.

#### IV. THE IMPORTANCE OF NUTRITION THROUGH THE VESSELS OF THEBESIIUS AND THE CORONARY VEINS IN CERTAIN PATHOLOGICAL STATES OF THE HEART.

There are three pathological states upon which the modes of nutrition under discussion appear to have an important bearing; namely, fibrillary contractions, arrest of the heart without fibrillation, and arterio-sclerosis of the coronary arteries.

The recovery of the cat's heart from fibrillary contractions has already been mentioned in the description of my experiments of April 1 and June 26. In one of these the heart was fed from the



FIGURE 5. Curve drawn immediately after marked fibrillary contractions, by an ordinary muscle lever attached to the apex of a cat's heart fed through the coronary sinus, June 26, 1897. The fibrillation took place one hour after excision of the heart, and lasted two minutes. The lower curve marks the time in seconds.

ventricle through the vessels of Thebesius; in the other, from the coronary sinus through the coronary veins. It is probable that nutrition through these channels is of great value to the struggling heart, both in preventing fibrillation, and possibly in recovering the heart after fibrillation has set in. That such a recovery may occur, even in the dog, has recently been demonstrated by MacWilliam<sup>1</sup>

<sup>1</sup> MACWILLIAM: *Journ. of Physiology*, 1887, viii, p. 299.

and Porter.<sup>1</sup> There is therefore no ground for denying the possibility of recovery in the human heart, although it is likely enough that such instances are extraordinarily rare; and if the cat's heart can be recovered by feeding through the vessels of Thebesius or the coronary veins, the importance of these modes of cardiac nutrition in the fibrillar contractions of the hearts of other mammals can hardly be gainsaid.

It should be remarked that the heart in the living animal is, during fibrillation, in a state particularly favorable to nutrition through both the vessels of Thebesius and the coronary veins. Measurements taken in the left ventricle show that the intracardiac pressure rises as fibrillation draws near,<sup>2</sup> so that the heart is greatly distended even before it has ceased to beat. The arrest of the ventricle lowers the blood-pressure in the aorta, and hence the blood-pressure in the smallest peripheral coronary arteries, to almost nothing. Consequently the passage of the blood through the vessels of Thebesius and the regurgitation from the auricle through the coronary veins are doubly aided; on the one hand by the relatively high pressure in the ventricle and auricle, and on the other by the diminished resistance in the coronary vessels. I have already demonstrated how slight a pressure will drive the blood from the interior of the ventricle or auricle through the cardiac walls. The intracardiac pressure at the onset and in the earlier moments of fibrillation would seem to be more than sufficient to establish such a circulation, giving the quivering organ one chance of recovery, although a desperate chance at best.

In simple arrest of the heart without fibrillation the nutrition through the vessels of Thebesius and the coronary veins may work to great advantage. Here once more the heart is distended; here again the pressure in the coronary arteries has fallen very low: but no fibrillation dissipates the energy of the cardiac muscle in futile, uncoördinated contractions. The occasional recovery of these arrested hearts can scarcely be explained by the theory that leaves the heart un-nourished save through the coronary arteries. It is the pressure in the aorta that drives the blood in the coronary arteries into the cardiac walls in spite of the peripheral resistance. The aortic pressure is maintained by the beat of the ventricle; the loss of a few successive beats lowers it enormously: the reservoir receives

<sup>1</sup> PORTER: *This Journal*, 1898, i, p. 71.

<sup>2</sup> PORTER: *Journ. of Physiol.*, 1894, xv, p. 133.

nothing, and is speedily drained through the smaller arteries into the capillaries and veins. The coronary capillaries receive their portion with the rest; but whence shall they get more? The pressure in the aorta has fallen, but the peripheral resistance in the coronary system still remains. The power that drove the blood through this resistance is gone, and the capillary areas once fed through the coronary arteries are thereby closed; they can be opened only by the forcible contraction of the left ventricle. But the ventricle lies passive; and should remain so, according to the prevailing belief that the heart is self-nourished and must beat or starve. It is here that the nutrition through the vessels of Thebesius and the coronary veins becomes of importance. The heart *is* self-nourished, but not through its arteries alone; the prostrate ventricle, although it has ceased to feed itself through the coronary arteries, can still be fed by the blood that distends its cavities, and by this endocardiac nutrition may gather strength to resume its load.

Still more interesting is the relation of endocardiac nutrition to arterio-sclerosis of the coronary arteries. The pathologist often finds the coronary arteries thick and stiff with calcareous deposits, their lumen greatly reduced or even wholly gone. And when he looks for the infarcts that should have followed the blocking of the terminal artery, he sometimes sees none. The area formerly supplied by the closed artery is occasionally apparently healthy. Life has continued for months or even years with what seems an impossible heart. No wonder that many anatomists and physicians still contend in the face of conclusive experiments that the coronary arteries are not truly "terminal." How else can such immunity from infarction be explained? What but a collateral circulation through branches freely communicating with other coronary arteries could have kept the ever active muscle from decay? The failure of the distal end of a severed coronary artery to bleed in the profuse way that indicates a free communication with other vascular areas; the fact that infarcts frequently, though not invariably, follow the embolism or thrombosis of these vessels during life; and, most conclusive of all, the easy production of infarcts by the ligation of coronary arteries,<sup>1</sup>—have not convinced some minds. They cling to the occasional freedom from infarction after thrombosis or embolism, and not seldom attempt to strengthen their position by

<sup>1</sup> KOLSTER: *Skandinav. Arch. f. Physiol.*, 1893, iv, p. 1; also PORTER, *Arch. f. d. ges. Physiol.*, 1893, lv, p. 366.

pointing to the fact that one coronary artery can be injected from another.

These writers forget what a terminal artery really is. They forget that terminal arteries, like all other blood-vessels, communicate with their neighbors by capillaries. An artery is terminal, not because it has no communication with neighboring arteries, but because this communication is of a particular kind. "Terminal" means simply that the resistance in the anastomosing branches is greater than the blood-pressure in the arteries leading to these branches. It is this resistance which makes the artery terminal. The concept is physiological, and only secondarily anatomical. The fact that an artery can be injected post-mortem from another artery is no evidence that the living blood in the living organ follows the course of the post-mortem injection. The natural relation between the blood-pressure in the arterioles and the resistance in the communicating vessels cannot be imitated with accuracy. Only injections that pass with great ease can be used as presumptive evidence against the terminal nature of the artery; and, as a fact, aside from rare abnormal cases, injections pass from one coronary artery to another with difficulty or not at all. The advocates of the free anastomosis of the coronary arteries have indeed a difficult position. They must explain how infarcts can follow the closure of freely anastomosing vessels. We who believe in the terminal nature of these arteries need only explain why the closure occasionally fails to produce infarction. And this explanation can now be given.

A certain small number of the cases in which closure fails to produce infarction must be ascribed to the abnormal anastomoses that are occasionally present. It is possible also that the very gradual closure of an artery might permit the gradual dilatation of the communicating vessels until the resistance in them is low enough to divert a part of the blood in the neighboring areas into the anæmic district, and thus gradually establish sufficient collateral circulation to keep the part alive. These possibilities have long been recognized.

A new and effective mechanism for the rescue of the cardiac muscle from threatened infarction is found in the nutrition through the vessels of Thebesius and the coronary veins. Through these ever present channels blood can be drawn to the anæmic area for the occasional saving of hearts in which the blocking has been sufficiently slow. Usually, however, the blood must find the resist-

ance in the arterial capillaries too great to be overcome, and will fail to prevent infarction.

It is evident that the nutrition through the vessels of Thebesius and the coronary veins must modify in no slight degree the existing views of the nutrition of the mammalian heart, and of the manner in which infarction of the heart takes place.

This work was undertaken at the suggestion of Dr. W. T. Porter. I wish, in concluding, to express my grateful appreciation of his encouragement and aid.

SUMMARY.

(1) The vessels of Thebesius open from the ventricles and auricles into a system of fine branches that communicate with the coronary arteries and veins by means of capillaries, and with the veins — but not with the arteries — by passages of somewhat larger size.

(2) These vessels are capable of bringing from the ventricular cavities to the heart-muscle sufficient nutriment to maintain long-continued, rhythmic contractions.

(3) The heart may also be effectively nourished by means of a flow of blood from the auricle back into the coronary sinus and veins.

(4) Nutrition through the vessels of Thebesius and the coronary veins contributes to the recovery of the heart from fibrillary contractions and from simple arrest without fibrillation, and affords a reasonable explanation of many cases in which the cardiac tissues have survived for months or even years the closure of terminal arteries long believed to be their sole supply.

# ON THE RELATION BETWEEN THE EXTERNAL STIMULUS APPLIED TO A NERVE AND THE RESULTING NERVE IMPULSE AS MEASURED BY THE ACTION CURRENT.

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THE experiments described in this paper were undertaken with the idea of ascertaining the quantitative relation between the strength of the external stimulus applied to the nerve, and the strength of the resulting nerve impulse. The nature of the nerve impulse is as yet undetermined. We may recognize its presence in the nerve, however, in two ways: by the change in the electrical condition of the nerve fibres which accompanies the nerve impulse, or by the changes produced in the peripheral organ in connection with the nerve. In the latter case the quantitative changes in the peripheral organ obey laws peculiar to the organ itself, and cannot, therefore, be used directly as an index to the quantitative changes in the nerve fibres.

On the other hand, the changes in the electrical condition of the nerve are immediately associated with and are presumably the direct result of the changes constituting the nerve impulse. The intensity of the electrical change is measurable in the form of the action current. In the experiments described in this paper the action current is taken throughout as a measure of the intensity of the nerve impulse, it being assumed, in accordance with recent authors,<sup>1</sup> that the action current varies proportionately with the change constituting the nerve impulse.

In support of this assumption we may refer especially to the work of Waller, who has made extensive use of the action current in the study of the reactions of nerve fibres to anæsthetics and to many of

<sup>1</sup> A. D. WALLER: *Brain*, xviii and xix; and *Journal of physiology*, xviii, p. xxxviii.

BIEDERMANN: *Electrophysiologie*, p. 658, E. STEINACH: *Pflüger's Archiv.*, 1894, lv, p. 487.



the chemical reagents used in experimental physiology and in medicine. His work offers strong presumptive evidence in favor of the view that there is a direct relation between the action current and the nerve impulse.

Waller also made observations on the relation between the strength of the external stimulus and the resulting electrical change in the nerve. He reached conclusions which will be discussed later in the paper.

#### METHODS AND APPARATUS.

Determinations of the quantitative relation between the strength of the external stimulus applied to a nerve and the strength of the resulting nerve impulse were made on isolated nerves from the frog (*Rana virescens*), the terrapin (*Pseudemys*, two species), the cat, and the dog. The particular nerves used were the sciatic, ulnar, and vagus.

Isolated pieces of nerve five to six centimetres in length were used for experimentation. During an experiment the nerve was laid across two pairs of nonpolarizable electrodes in a moist chamber, one pair of the electrodes being used to stimulate one end of the nerve, the other pair to lead off the action current from the other end of the nerve to a very sensitive aperiodic galvanometer.

The form of stimulus used throughout the experiment was the induced current produced by a du Bois-Reymond induction coil. The secondary circuit included an automatic short-circuiting key, the stimulating electrodes, and an electro-dynamometer. The short-circuiting key consisted of a small clock placed horizontally and carrying on its second-hand a platinum connector which revolved in two parallel circular mercury troughs. One of these troughs was interrupted for a definite proportion of its circumference.

The intensity of the stimulus was varied by moving the secondary coil of the induction apparatus toward or away from the primary coil, according to the maker's scale of proportional currents, the intensity of the resulting current being measured by the electro-dynamometer in the circuit. The primary circuit was fed by a large storage cell with sufficient resistance in the circuit to give a current of the desired intensity. This arrangement gave a practically constant current during the time limits of an experiment. The current was interrupted by the Neef's hammer attached to the induction coil. Helmholtz's modification was used for equalizing the make and the break inductions.

The electrical measuring instruments used in these experiments are new and especially adapted to physiological work. They may, therefore, be given special mention. Both instruments were devised by Professor H. A. Rowland of the Johns Hopkins University.

The galvanometer is of the d'Arsonval type. It consists of a small coil of fine wire suspended between the closely approximated poles of a powerful horse-shoe magnet. The coil is suspended by a phosphor-bronze filament and the circuit is completed by a spiral spring of the same material at the bottom of the coil. The instrument is rendered aperiodic by a mica vane attached to the suspended coil and swinging in an enclosed box. The deflections of this movable coil are determined by means of a telescope reading the divisions of a scale reflected from a mirror attached to the coil. The reading telescope is attached to the instrument by means of a horizontal arm which can be raised or lowered.

The galvanometer is independent of the earth's magnetism and can therefore be placed upon any convenient supporting surface without reference to the magnetic meridian. When the instrument is once adjusted it is always ready for immediate use, and its sensitiveness, the ease and accuracy with which its deflections may be read, and its freedom from vibrations, make it very convenient in physiological experimentation.

The particular instrument used in these experiments has a resistance of 671 ohms, and gives a current value of 49 ten-millionths of a milli-ampere ( $49 \times 10^{-10}$  amperes) per millimetre of scale deflection.

The electro-dynamometer consists of a small high resistance coil of fine wire suspended within and at right angles to a fixed coil of high resistance. The free coil is suspended in precisely the same manner as in the galvanometer just described. The instrument is rendered aperiodic by a mica vane, and the deflections are read by means of a telescope and scale, as in the galvanometer. The magnetic field in this instrument is produced by passing the current to be measured through the fixed coil. A current sent through both coils gives a deflection in a constant direction, whether the current be direct or rapidly reversed. It is therefore especially adapted to the measurement of alternating induction currents, such as are used in physiological experiments.

The deflection of the suspended coil in this instrument represents the mean squares of the series of waves of induced current, hence the mean current varies directly as the square root of the deflection.

The resistance of the electro-dynamometer used is 3371 ohms. Measured by direct current methods it gives a standard value of 158 ten-thousandths of a milli-ampere ( $158 \times 10^{-7}$  amperes) for the unit obtained by taking the square root of the scale deflection measured in millimetres. It must be remembered, however, that this value represents only the mean value of the series of waves of induced current as stated above.

To those accustomed to use induction currents for physiological stimulation, it will be of additional value to know that 2.5 units of current as measured on this electro-dynamometer can just be detected by the sensory effect on the tip of the tongue, that 6 to 8 units become sharp, and 12 units painful.

The range of stimulation strength ordinarily used in the laboratory varies between 5 and 10 units of current as defined above. The range of the dynamometer extends from 0.5 to 15 units. This range just about covers the working range of stimulating currents, though scarcely delicate enough to measure minimal stimuli for very sensitive nerves, such as those of winter frogs.

In each experiment the nerve was stimulated for 12 seconds, once every minute, until the desired variation of strength of stimulation was obtained. The resulting action currents were led from the nerve to the galvanometer for measurement. The time of the stimulation was regulated by the automatic short-circuiting clock key already described. The strength of the stimulus was varied by moving the secondary coil toward or away from the primary in the interval between stimulations.

In some control experiments records of the muscular contractions were also taken. In these experiments the gastrocnemius muscle was left in connection with the sciatic nerve and arranged in the usual way in the moist chamber, the muscle lever recording on a slowly moving drum. The sciatic nerve was stimulated near the muscle and the resulting action current was led off from the central end of the nerve to the galvanometer. Experiments of this kind were made on preparations from the frog and the turtle, but not on the nerves from mammals. However, in most experiments the isolated nerve alone was used.

#### THE RESULTS OF EXPERIMENTS.

The quantitative relation between the strength of the external stimulus applied to the nerve and the strength of the action current

produced by it, is shown in the curves constructed from the experiments. In these curves I have plotted units of increase of stimulating current along the abscissæ and the associated change in the strength of the action current along the ordinates.

One millimetre of scale deflection of the galvanometer by the action current has a value of 49 ten-millionths of a milli-ampere, as previously stated. There is a great difference in the absolute values of the action currents obtained from different nerves, but as we are concerned here only with the relative variations in values I have reduced all curves to a uniform magnification in order to facilitate comparisons.

**Nerves of the Frog.**—The plotted curve expressing the relation between stimuli of different strengths applied to a frog nerve and the strengths of the resulting action currents shows three characteristic parts (Fig. I.): (1) an abruptly ascending straight limb, including the range from minimal nerve stimuli to stimuli above the strength that calls forth maximal muscular contractions; (2) a middle portion, in which the curve is still ascending but strongly concave to the abscissa; (3) a supra-maximal straight portion parallel to or more often slightly diverging from the abscissa.

These regions I shall now describe in greater detail. The first record of a current of action in a frog nerve was obtained when the nerve was stimulated with an induced current just strong enough to produce a minimal muscular contraction. This was the usual result in test experiments, although sometimes the minimal muscular contraction was obtained with a stimulus slightly weaker than that causing a readable action current in the nerve. In every case the minimal action current produced in the galvanometer a deflection of only a fraction of a millimetre.

A series of stimuli applied to the nerve, increasing above the minimal stimulus by very small but equal increments up to a strength about twice that necessary to call forth a maximal muscular effect, produced a relatively strong and rapid increase in the action current. This increase in the action current was by increments proportional to the increase in stimulus, giving in the plotted curve a straight line. Such slight variations as occurred in successful experiments were apparently within the limits of error of determination. The strength of a minimal stimulating current for the nerve varied from 0.02 to 0.2 units. The increase in stimulus that was necessary in order to call forth maximal muscular contractions in test experiments, was approxi-

mately 0.2 of a unit. The upper limit of the first straight portion of the curve is not sharply bounded.

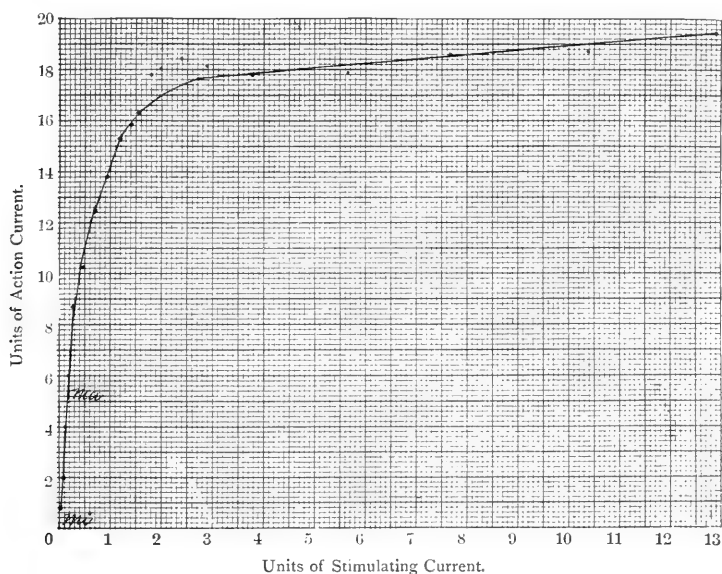


FIG. 1. SCIATIC OF THE FROG. EXPERIMENT 39.

Curve representing the quantitative relation between the strength of an external stimulus applied to the frog's sciatic and the strength of the resulting action current. The abscissæ represent units of stimulating current (1 unit =  $158 \times 10^{-7}$  amperes). The ordinates represent units of action current (1 unit =  $49 \times 10^{-10}$  amperes). The dots on the plot represent the individual readings. The mean for this series of readings was drawn after a comparison of several typical experiments.

Mi. Minimal muscular effects. Ma. Maximal muscular effects.

A further increase in the strength of the stimulus above that just described produced an increase in the action current of the nerve, but the added increments of action current accompanying the increase in stimulus were successively smaller. At this point the plotted curve is strongly concave to the abscissa. This middle portion of the curve of relation between the strength of the stimulus and the strength of the action current varies in extent and in degree of concavity, and its limits can be determined only very roughly.

A further increase in the strength of the stimulus produced only a slight additional increase of the action current, even though the strength of the stimulus was increased tenfold and more. This slight

increase in the action current was by increments proportional to the increase in stimulus. When plotted, the results give a straight line slightly diverging from the abscissa. Occasionally there is no increase in the amount of the action current, and the line is then parallel to the abscissa.

The nerves of winter frogs are sometimes very irritable and the relations of minimal stimuli to the corresponding action currents are accordingly difficult to determine. The strength of minimal stimuli for many frog nerves was too slight to be measured by the electro-dynamometer, and in such experiments it was determined indirectly by the corrected scale of the induction coil.<sup>1</sup> In these very irritable nerves the abruptness in the increase in action current with small increase above minimal stimuli is most marked. Variations and irregularities were frequently obtained also in the response of frog nerves to stronger stimuli.

**Nerves of the Terrapin.**—Nerves of the terrapin (*Pseudemys*), are much less irritable than those of the frog, but the curve of relation between stimulus and action current response, plotted from experiments on the sciatic of the terrapin, exhibits the general characters just described for that of the sciatic of the frog. That is, it shows a beginning straight limb of rapidly increasing action current produced by slight increase in the energy of the stimulus, a middle portion concave to the abscissa in which the action current still increases, but by diminishing increments, and a long supra-maximal straight limb slightly diverging from the abscissa.

The first straight part of the curve usually ascends less abruptly from the abscissa and the second curved portion is usually longer and less concave than in the curve from the frog's sciatic. The series of tests as a whole was more regular and constant than in the experiments on the frog's sciatic.

The total action current obtained from the nerves of the terrapin was relatively much less than that obtained from the frog's sciatic. A minimal stimulus for the sciatic of the terrapin varied from 0.7 to 2 units of stimulus; an increase above this by 0.3 to 0.7 units produced a maximal stimulus for the muscle attached to the nerve in test experiments.

<sup>1</sup> By a new arrangement of the dynamometer, suggested by Professor Rowland, this difficulty has been obviated, the sensitiveness of the dynamometer being so greatly increased that minimal or sub-minimal stimulating currents, even for very irritable nerves, can probably be determined without difficulty.

The results of experiments on the vagus of the terrapin have been quite variable. In eight experiments no action current was obtained from the vagus, even with excessively strong stimuli. In other experiments, especially after the nerves were kept in the moist chamber for from one to three hours, an action current of relatively slight intensity was obtained. The results of experiments which yielded the most pronounced action currents, when plotted, exhibit in a degree the relations shown by the curve from the sciatic of the terrapin. However, the first straight portion of the curve is obscure in experiments on the vagus in that it merges at once into the second or strongly concave portion. The upper straight limb of the curve is quite closely comparable to that in curves from the sciatic nerve.

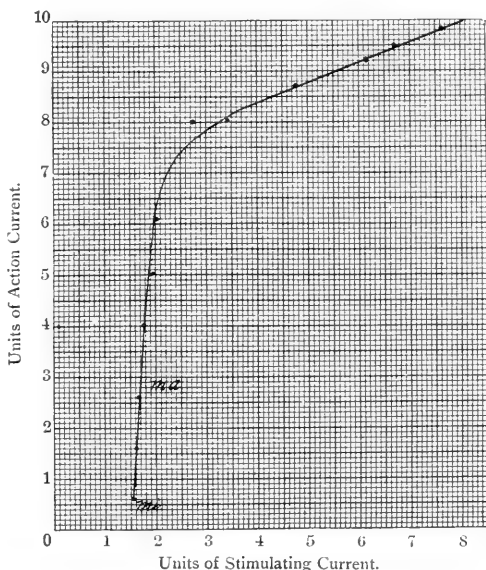


FIG. 2. SCIATIC OF THE TERRAPIN. EXPERIMENT 102.

Curve representing the quantitative relation between the strength of an external stimulating current applied to the sciatic of the terrapin and the strength of the resulting action current. As in Fig. 1, the abscissæ represent units of stimulating current, while the ordinates represent units of action current. The upper limb of the curve in this experiment diverges from the abscissa more than does the average of the whole set of experiments.

Mi. Minimal muscular effects. Ma. Maximal muscular effects.

The striking difference in irritability of nerves from the frog and from the terrapin is shown by Figs. 1 and 2.

**Nerves of the Cat and the Dog.** — Nerves of the cat and the dog were used under precisely the same conditions as those of the frog and the terrapin. The curve expressing the relation between the strength of the external stimulus and the strength of the action current in nerves of the cat and the dog (Figs. 3 and 4) is very like that obtained from the frog or the terrapin. Beginning with minimal stimuli applied to the nerve and increasing by small but equal increments of stimulation there is a rapid increase in the amount of the action current also by equal proportional increments.

This ascending straight limb of the curve is followed by a middle portion increasing by diminishing increments and thus concave to the abscissa, and this, finally, by a long supra-maximal straight portion diverging slightly from the abscissa.

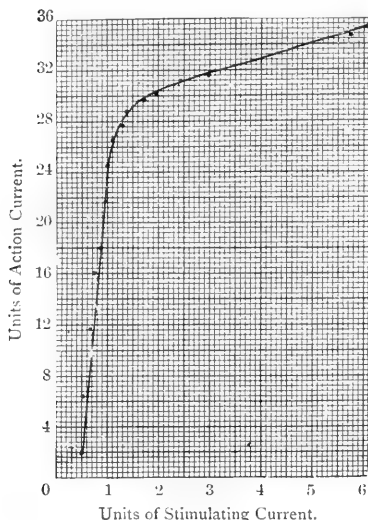


FIG. 3. ISOLATED SCIATIC OF THE DOG.  
EXPERIMENT 106.

Showing the relation between the strength of an external stimulus and the strength of the resulting action current. Units of stimulating current along the abscissæ, units of action current along the ordinates.

For the full extent of the upper straight limb of the curve, compare with Fig. 4. This experiment was performed after the nerve had been kept alive in the moist chamber at room temperature for 16 hours.

The middle portion of the curve is usually longer and less concave (Fig. 4, *ab*) than in the curves from the nerves of the frog or the terrapin, but sometimes this difference is not apparent. In a small percentage of experiments on the sciatic of the dog I have obtained an initial short curve convex to the abscissa. That is, with an increase by equal increments above a minimal stimulus for the nerve there is for one or two readings an increase in the action current by successively increasing increments. This short initial part of the curve is always followed by the first straight portion as described above. It should be stated here that the long and tedious precautions for cooling the animal to room temperature before its death, as described by Bernard, Schiff, and Israel,<sup>1</sup> seemed unnecessary and were not observed. The animals used were such as had been killed in the laboratory at the end of other experiments. The nerves were taken soon after the animal was killed in

some instances, and in others not until the animal had cooled to near the room temperature. In one case, one sciatic of a dog was removed at once after the animal was killed, the other after it had cooled to near the room temperature. No difference was observed between the character of the results in experiments made on the two nerves immediately thereafter. However, after being kept over night on clean filter paper moistened with physiological saline solution, the

<sup>1</sup> OSCAR ISRAEL: Arch. für Anat. u. Physiol. (physiol. Abth.), 1877, p. 443.



warm nerve gave no response even to the strongest stimuli, while the cooled nerve was still alive as shown by the strong action current. A nerve taken from the body of a dog after 15 hours, was dead, while nerves from the same dog removed immediately and kept on filter paper for 15 hours were still alive and very irritable, as shown by the marked action current caused by stimulation.

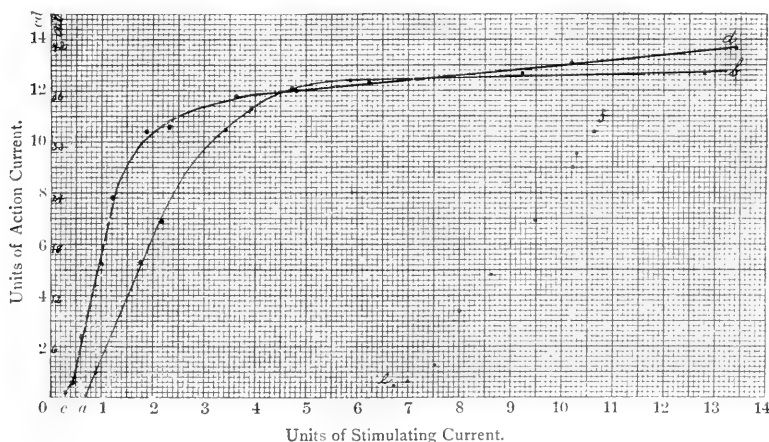


FIG. 4. SCIATIC OF THE DOG. EXPERIMENTS 91, 95, AND 118.

Curves expressing the relation between the strength of stimulating current and the resulting action current. No. 91, *ab*, from the nerve immediately after its removal from the body. No. 95, *cd*, from the same nerve after 17 hours in a moist chamber at room temperature. The curve *cd* shows in comparison with the curve *ab* the increase in irritability and decrease in the amount of the action current which occurs when nerves are kept for some time. No. 118, *ef*, the beginning of a curve introduced here to show the initial minimal portion convex to the abscissa. Curve *cd* also shows the phenomenon. The values of the stimulating current and the action current are not given in curve *ef*, but the magnification is about twice that of the curves *ab* and *cd*.

Nerves from dogs and cats remain alive in the moist chamber at room temperature a surprisingly long time. Nerves from the dog have been frequently kept alive over night and experimented on the next day 15 to 20 hours after removal from the body of the animal. In one case two sciatics and an ulnar of the dog were alive and hyper-irritable after 25 hours, and the following morning, after an isolation of 41 hours<sup>1</sup> at room temperature, the ulnar still gave a small action current; the two sciatics gave no action current, *i. e.*, were no longer

<sup>1</sup> L. Fredericq obtained an action current after 24 hours. Archiv. f. Anat. u. Physiol., 1880, p. 70.

capable of responding to stimuli. Even in these sciatics, however, very strong currents of rest were present.

I have uniformly obtained a strong action current from isolated nerves of the dog. The action current decreases after 12 hours (see Table), but a current may still be obtained after at least 41 hours. The action current varied from 20 to 30 per cent of the demarcation current in fresh nerves. The strongest action current Grützner<sup>1</sup> obtained from rabbit nerves did not exceed 4 per cent of the demarcation current. As the nerves were kept, the demarcation current increased for some time, becoming two to three times the original value after 15 hours, — the measurement being made after a fresh section of the nerve. This increase in the value of the demarcation current was true also for the nerves of the terrapin, and has been described before in the nerves of frogs.<sup>2</sup>

TABLE

*Showing data from successive experiments on the same nerve, to illustrate the increase in irritability and the decrease in maximal action current, which occur when the nerve is kept alive on filter paper wet with physiological saline solution.*

FROM THE ULNAR NERVE OF THE DOG.

ULNAR OF DOG.	Strength of Stimulating Current giving rise to a minimal action current.	Strength of Action Current with a supra-maximal stimulus, 10 units.
After 20 minutes.	1.94 units.	33.2 mm. of deflection.
" 18 hours.	.54 "	17.4 " "
" 24 "	.25 "	9.8 " "
" 41 "	.55 "	0.9 " "

The irritability of isolated nerves from the cat and the dog increased for several hours, 24 hours at least, and then decreased until its final disappearance. The test on which this statement is based is the strength of a minimal stimulus necessary to produce an action current in the nerve.

#### COMPARISON OF RESULTS.

From about one hundred and twenty experiments I am unable to deduce any general law applicable to the entire curve of relation

<sup>1</sup> GRÜTZNER: Pflüger's Archiv., 1881, xxv, p. 278.

<sup>2</sup> HOWELL: Journal of physiology, 1894, xvi, p. 476.

between the external stimulus applied to a nerve and the strength of the action current produced. There is a close similarity in the form of the curves expressing the results of experiments on nerves from the three classes of animals used. In every case the entire curve shows three distinct parts as described above. Each of these parts varies according to its own laws. The first part and the last portion vary according to arithmetical ratios, but the ratios are different in the two portions.

Waller,<sup>1</sup> from his experiments, describes the relation of the strength of the external stimulus and the action current response as an S-shaped curve consisting of three portions: (1) an initial "short subminimal portion convex to the abscissa, increasing by increasing increments; (2) a long straight middle portion, inclusive of and beyond the functional range of nerve measured by maximal muscular effects, increasing by equal increments; (3) an ultra-maximal portion far above the values of maximal functional effects concave to the abscissa, increasing by diminishing increments."

The first sub-minimal portion of the curve that he describes I have been unable to obtain on the nerves of the frog. But on the sciatic of the dog I have obtained a short initial curve similar to that described by Waller for the nerves of the frog, although I cannot say what relation it bears to minimal muscular contractions. This initial curve in the nerves of the dog, when present, is much more strongly marked than in the single curve which Waller shows. In fact, the particular experiment that he has plotted exhibits a variation scarcely greater than might be accounted for by the limits of error in determination. However, some of his tables,<sup>2</sup> if plotted, show the phenomenon much more strongly, while others do not exhibit it at all.

My results on all nerves confirm Waller's statements with reference to the second part of the curve which he describes. That is, I have found that variations in the strength of the stimulus between the limits of strength that call forth minimal and maximal muscular contractions are accompanied by proportionately increasing increments of action current. In experiments in which I have used the nerve alone, this region is a straight line in the plotted results. Waller's supra-maximal portion of the curve seems to correspond with the

<sup>1</sup> WALLER: *Brain*, xviii, p. 200.

<sup>2</sup> WALLER: *Journal of physiology*, 1895, xviii, p. xxxii, Exps. 103, 130a, 130b.

middle portion of my curves, which, following his description, I have spoken of as concave to the abscissa.

The third or supra-maximal straight portion, which I have described and figured, he does not describe, although the data for it is contained in one of his protocols (No. 106). Throughout this portion of the curve a wide variation in the strength of the stimulating current occasions but little and in some cases practically no variation in the strength of the action current. We have here a phenomenon which at first sight suggests a comparison with the maximal contraction of a muscle. This maximum is reached, in the nerve, at a point far beyond the strength necessary to cause a maximal muscular contraction. It lies, therefore, beyond the probable range of normal functional activity. As between the muscle and the motor nerve, however, it may be said that the latter is capable of imparting a stimulus far in excess of that necessary for the production of a maximal shortening in the muscle. How completely this relation may hold when the nerve fibre is stimulated from its cell instead of directly by artificial means, cannot be determined.

Finally, I wish to express my deep obligation to Professor W. H. Howell, at whose instigation this work was undertaken. To his many helpful directions and suggestions given while prosecuting the investigation, and especially to his kindly criticism during the preparation of the manuscript, are chiefly due whatever of value attaches to this paper.

## ON THE NATURE OF THE CARDIOPNEUMATIC MOVEMENTS.

By S. J. MELTZER.

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FOR more than thirty years it has been known that the air in the respiratory tract shows slight periodical oscillations, synchronous in rhythm and time with the cardiac beats, — the cardiopneumatic movements, as they have been called since Landois first gave them the name.

C. Voit<sup>1</sup> observed these movements while breathing through a Müller's valve. During a respiratory intermission in which the glottis remained open and the nose closed, regular oscillations were noticed consisting of tiny inspirations and expirations. Voit found the inspiratory motion synchronous with the systole, and the expiratory with the diastole of the heart. He explained the phenomenon by the assumption that each systole — diminishing the volume of the heart — causes a lowering of pressure within the thoracic cavity, and thereby an aspiration of air by the lungs, — an inspiration; while the diastole increases the pressure, and causes an expiration.

Two years later Terné van der Heul,<sup>2</sup> under the direction of Donders, attempted to get tracings of these movements as they are obtainable from the nose, and was surprised to find that, in his case, the inspiration coincided with the diastole and the expiration with the systole, the tracing presenting a regular sphygmogram. It is probable, however, that in van der Heul's case the glottis was closed while the tracings were taken, these therefore being in fact nothing but sphygmograms, *i. e.*, the variation in the volume of blood within the closed cavity of the nose. Many persons, as Mosso has shown, are indeed unable to keep the glottis open while they stop breathing. The writer of this article can witness to that fact. In the few attempts which he has made to obtain cardiopneumograms from the nose the result has been invariably a sphygmogram.

<sup>1</sup> VORT: *Zeitschrift für Biologie*, 1865, i, p. 390. The phenomenon had already been described before Voit by Buisson, *Gaz. med. de Paris*, 1861, p. 320.

<sup>2</sup> TERNÉ VAN DER HEUL: *Nederlandsch Archief voor Genees- en Natuurkunde*, 1867.

Ceradini<sup>1</sup> studied the movements at first by means of a manometer connected with the nose or mouth, and containing smoke or colored liquid; he confirmed the statement of Voit, and also adopted his explanation of the phenomenon. In a later study,<sup>2</sup> however, Ceradini modified his first interpretation. He came to the conclusion that the systolic condition of the heart could not change the pressure within the thoracic cavity as long as the blood put out from the left ventricle is still within the cavity. Ceradini, therefore, now assumed that the oscillations did not originate from the change in the volume of the heart, but from the change in the volume of blood present in the thoracic cavity; the inspiration being due to the outflow from the cavity of the chest, and the expiration due to the inflow of blood into the cavity. Ceradini also obtained tracings of these movements by means of an instrument which he called "Hæmothorakographie,"<sup>3</sup> but never published them.

In 1876 Landois<sup>4</sup> published an elaborate monograph on these oscillations, which he termed, as stated above, cardiopneumatic movements. He studied the movements with the aid of the manometric flame, and, also, by means of his cardiopneumograph, obtained tracings of them from the nose and mouth of men and from the trachea of curarized dogs. The skeleton of his tracings shows a sudden steep inspiratory, and a gradual, slanting expiratory undulation, which Landois, adopting Ceradini's later view, explains by the sudden arterial output of blood from, and the gradual venous flow of blood into, the thorax. Besides these main movements, Landois noticed on his tracings little secondary notches and peaks which he attempted to explain by certain subsidiary but constant causes. The rebound of the arterial blood causes a tiny peak on the expiratory line. Landois insists that each inspiratory movement is preceded by a small sharp expiratory motion, which he explains by an assumption that I shall not discuss here. Mosso,<sup>5</sup> on the other hand, who accepts the views of Ceradini and Landois with regard to the chief cause of these movements, claims that no short respiratory upstroke precedes the inspiratory movement, and that, in his opinion, the beginning of this latter

<sup>1</sup> CERADINI: *Heidelberger Jahrbücher der Literatur*, 1869, p. 912.

<sup>2</sup> *Ibid*: *Annali universali di medicina*, 1870, p. 587.

<sup>3</sup> This instrument, as well as the cardiopneumograph of Landois, is practically nothing more than a Marey's tambour.

<sup>4</sup> LANDOIS: *Graphische Untersuchungen über den Herzschlag*. Berlin, 1876.

<sup>5</sup> MOSO: *Die Diagnostik des Pulses*, 1879.

movement is caused by the widening of the chest due to the heart impulse.

Finally, Martius, who wrote a very elaborate article on the "cardiopneumatic movements in the œsophagus," should also be mentioned, but I shall not discuss his article here. I wish only to say that Martius, in his very elaborate analysis of his tracings, also adopts the view that the flow of blood into and out of the thorax is the chief cause of the cardiopneumatic undulations.

Thus, the authors mentioned, and many other writers<sup>1</sup> on the cardiopneumatic movements, all agree, so far as I can see, that the movements are expressions of the variations of pressure within the air-tight thoracic cavity caused by changes either in the volume of the heart or in the volume of the blood contained in the thoracic cavity.

A recent article by Haycraft and Edie<sup>2</sup> opposes this view. These authors state that the cardiopneumatic movements "occur equally well with the chest cavity open and freely communicating with the outer air" (p. 429). It is obvious, therefore, they say, that the hypothesis of Ceradini and Landois can hardly be accepted as explaining the cardiopneumatic movements. They offer another hypothesis instead, namely, that the movements are "simply due to the heart pressing against the lungs, and that the lungs acted like an oncometer placed around it" (p. 430). The proof for this hypothesis they see in the fact that the cardiopneumatic movements almost entirely cease on lifting the heart away from the lungs. The writers are so thoroughly convinced of the correctness and simplicity of their position that they can hardly understand why it was overlooked by all the other writers on the subject, especially by Landois, who was the first one to obtain tracings of these movements. "It is a pity that he did not go a step or two further," they say, "their entire explanation must have dawned upon him" (p. 435).

The purpose of this Paper is to test the validity of the views advanced by Haycraft and Edie. Before entering into a further discussion, I wish, however, to remark that identical views were

<sup>1</sup> LOVEN: Nord. med. Ark., 1870, no. 19; P. BERT: Leçons sur la physiologie comparée de la respiration, Paris, 1870, p. 388; VON BRUNN: Berliner klinische Wochenschrift, 1872, p. 130; REGNARD: Revue mensuelle de médecine et de chirurgie, 1877, p. 333; LÉPINE: *ibid.*, p. 394; FRANÇOIS-FRANCK: Travaux du laboratoire de Marey, 1877, p. 233.

<sup>2</sup> HAYCRAFT and EDIE: Journal of physiology, 1891, xii, p. 426.

advanced by Klemensiewicz<sup>1</sup> about fourteen years before Haycraft and Edie, just after the appearance of the monograph of Landois. The original article is not accessible to me, but there is a short report of it in the *Jahresbericht* of Hofmann und Schwalbe<sup>2</sup> which I will quote here in part. "Klemensiewicz reports experiments on the cardiopneumatic movements, which can also be seen in the frog. He rejects the explanation of Landois because the phenomenon is also present when the thorax is open. The movements are due rather to the intimate relation between the heart and the lungs, the latter, like a large Marey's tambour, registering all the movements and vibrations of the heart." We see here the same hypothesis as that of Haycraft and Edie, and also the same argument for rejection of the hypothesis of Landois and others. In discussing the new hypothesis and the arguments against the older one, I shall use the statements of Haycraft and Edie, as I do not know the experiments upon which Klemensiewicz has based his opinion.

According to Haycraft and Edie, the cardiopneumatic curve represents the variations in the pressure exerted by the heart upon the lungs. As the heart slowly changes its size during diastole, it presses upon the lungs and causes the gradual (expiratory) upstroke. In the beginning of the systole the heart at first "resents" its distortion by the lungs, — it "asserts" itself, which again causes a small but steep upstroke; then as it becomes smaller in all dimensions, it recedes from the lungs, which now expand, thus causing a sharp downstroke, — an inspiration. In a certain sense the theory of Haycraft and Edie coincides with the original hypothesis of Voit and Ceradini, inasmuch as both agree that the movements are due to the change of pressure caused by the change in the size of the heart; but while Voit and Ceradini meant the pressure exerted upon all the contents of the thoracic cavity, Haycraft and Edie mean only the direct pressure upon the lungs.

The interpretation of these authors, however, seems to me to be open to some criticism. We can understand why the pressure of the heart upon the lungs during diastole should produce an expiratory movement; we can admit the possibility that the "self-assertion" of

<sup>1</sup> KLEMENSIEWICZ, R.: Beiträge zur Demonstration des Pulses und Herzstosses mittelst der manometrischen Flamme, nebst Versuchen über die sog. cardiopneumatischen Bewegungen. Mitteilungen des Vereins der Aerzte im Steiermark, 1875-76.

<sup>2</sup> Jahresbericht über die Fortschritte der Anatomie und Physiologie, 1877, p. 56.



the heart will also cause a slight expiratory puff; but we cannot comprehend why the recession of the heart during systole should be followed by an inspiratory movement, — by an expansion of the lungs. Even in a state of collapse, the lungs are not perfectly atelectatic, but contain some air, and their elastic tissues are still stretched. When, now, air is driven out by the alleged pressure of the heart, expanding in diastole, what force is then present in the open chest that could distend the lungs anew against their own elastic tension, after they are released from the heart's pressure? In the tracings obtained from the lungs while the thorax is open, the apparent expansion of the lungs after the alleged diastolic pressure of the heart upon them has been withdrawn may be due to the elastic reaction of the rubber cover of Marey's tambour, which strives to return to its position of equilibrium after being stretched by the preceding expiration caused by the hypothetical pressure of the heart in diastole. But in the normal individual, in whom the elastic lungs are always considerably stretched, what could cause the lungs to expand again during systole against the strong pull of their elastic fibres; especially in such experiments as Landois and Ceradini made, in which the cardiopneumatic movements were demonstrated by the gas-flame and by smoke in a manometer? Smoke is lighter even than air, but, nevertheless, an inspiratory movement is seen in the smoke-column during each systole. What force except the negative pressure could compel the lung to follow the receding heart?

Again, Haycraft and Edie explain the entire cardiopneumatic curve also by the pressure of the heart upon the lungs, and do not permit the change in the pressure within the thorax to exert any influence upon it. But it is, nevertheless, an indisputable fact that during each cardiac cycle there is a considerable variation in the volume of blood present in the thorax. Now, why should this change of volume not cause a change of pressure in the normal, air-tight thorax, and why should this change of pressure not cause an expiration and inspiration, and leave a marked impression upon the cardiopneumatic curve? In this connection, it may be observed that Haycraft and Edie were not very fortunate in comparing the effect of the heart upon the lungs with the working of an oncometer. Here, too, the contractions of the organ enclosed within the oncometer are transmitted only by the changes in the negative pressure which they cause in the air-tight capsule of the instrument.

Furthermore, Haycraft and Edie in support of their statement that the open thorax has no influence upon the cardiopneumatic movements have published two figures, each containing two tracings, — one taken before and the other after the opening of the thorax. In both figures, however, the tracings taken when the thorax was open differ distinctly from those taken from the normal animal. I reproduce here one of these figures.

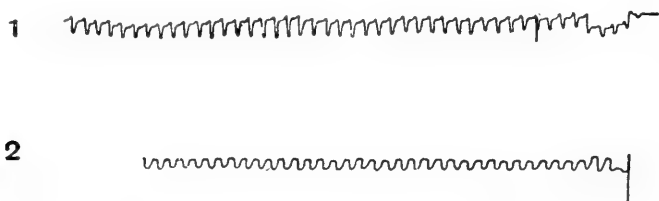


FIG. 1. (Fig. 4 in Haycraft and Edie's article; *Journal of Physiology*, 1891, xii, p. 431).  
 "Tracing (1) was taken from a rabbit with its chest cavity in normal condition; tracing (2) after making a free opening into the cavity."

In order to gain a personal insight into this problem I have repeated the experiments of Haycraft and Edie. These authors do not tell us directly from what animals they derived their results. There is a remark in their article that they have experimented with the lungs of rabbits and sheep, but this has reference to another kind of experiments. It is said, however, that the tracings which they present were obtained from rabbits. I have therefore first experimented on rabbits. The method was in general the same as that employed by the authors mentioned and by Landois. The animals were given chloral and then curare, and were kept alive by artificial respiration. The tracheal cannula was connected with a Y tube, each limb being provided with a stop-cock. One limb was connected with the apparatus for artificial respiration, and the other with a Marey's tambour registering on a revolving cylinder. When the stop-cock leading to the apparatus for artificial respiration was closed and that leading to the Marey's tambour opened, the cardiopneumatic movements soon appeared. After taking a few tracings from the normal animal, the chest was opened and tracings were again taken. I repeated these experiments on eight rabbits, from every one of which I uniformly obtained the following result: when both pleural cavities were freely opened there was no cardiopneumatic oscillation on the tracing from the trachea.

Fig. 2 shows a part of the tracings taken just before and after the opening of the thorax. The tracing taken soon after both sides of the thorax were opened is simply a straight line, drawn from a lower level on account of the collapsed condition of the lungs.

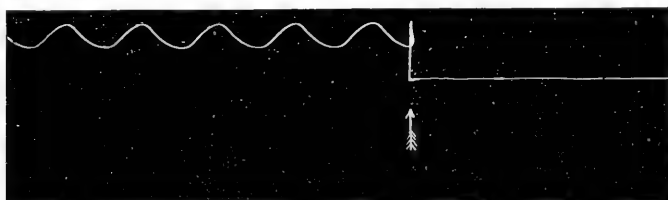


FIG. 2. The upper tracing is a cardiopneumogram from the trachea of a rabbit. On the left of the arrow are the cardiopneumograms taken while the chest was intact. After opening both pleural cavities all movements disappeared, and the tambour registered the straight line to the right of the arrow.

My results then were in direct contradiction to those described by Haycraft and Edie. I soon discovered, however, that there was a difference between the method of opening the thorax employed by Haycraft and Edie and that employed by myself. I usually opened each pleural cavity separately on their respective sides of the sternum. In one experiment in which I removed a part of the sternum some slight undulations could indeed be noticed on the tracing obtained from the trachea immediately after opening the chest (see Fig. 3).

Haycraft and Edie opened the abdominal cavity and cut away the anterior attachments of the diaphragm along with the anterior chest wall in its lower part. Of this method, which Haycraft calls the best,

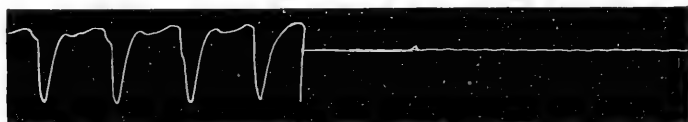


FIG. 3. Cardiopneumatic tracing, from a rabbit. At *x* a part of the sternum was removed; some fine undulations are noticeable on the line to the right.

he himself speaks as follows: "As soon as this is done, the heart will fall down in its natural bed formed posteriorly by the lungs and will be carried up and down by them with the movements of respiration; it will be loosened from its more solid moorings to the chest wall and will be seen floating as it were upon the lungs. It is in this position that physiologists from Harvey's time onwards have chiefly

studied its movements, making, I think, too little allowance for the fact that the organ is in an entirely unnatural condition in respect to its attachment."<sup>1</sup> I think that Haycraft is correct in this, but that he and Edie themselves make too little allowance for the fact that in their experiments the heart was in an entirely unnatural condition with respect to its attachments. I have repeated their experiment also on a number of dogs, and have convinced myself more than once that when the pleural cavities are opened without disturbing the natural relations of the heart to the front wall and diaphragm, the cardiopneumatic movements disappear either entirely or are hardly perceptible. When, however, as in Fig. 3, the attachments to the sternum and the diaphragm are removed and the heart allowed to fall back upon the lungs, some distinct movements appear again.

The experiment illustrated by Fig. 4 shows us (1) that the opening of the thorax, without disturbing the natural attachments of the heart, nearly destroys the movements (see tracings Nos. 2 and 4); (2) that the closure of the opening in the chest, though surely not absolutely air-tight, brings the movements out again to a certain degree (tracings Nos. 3 and 6); (3) that the removal of the normal attachments of the heart causes the heart to fall back upon the lungs and brings out movements that seem to be different in size and character from those which occur when the chest is air-tight and intact (tracing No. 5).

There is still another point which deserves special comment. If the communication between the trachea and the Marey's tambour were established immediately after the artificial respiration is discontinued, the rubber cover of the tambour would be exposed to an abnormally high pressure by the air escaping from the inflated lungs. To avoid this I have regularly permitted the thorough escape of the air from the lungs before a tracing was taken. To attain this I placed a **T**-tube between the stop-cock on the limb leading to the apparatus for artificial respiration and the tracheal cannula; this tube had a stop-cock on its vertical limb which was kept slightly open during the artificial respiration, and which I might call "the relief stop-cock." Before taking a tracing the following order was observed. First the relief stop-cock was opened fully, then the stop-cock of the artificial respiration was closed and that leading to the tambour was opened; and, finally, after the lever of the tambour reached its nor-

<sup>1</sup> HAYCRAFT, J. B.: The movements of the heart within the chest-cavity and the cardiogram. *Journal of physiology*, 1891, xii, p. 447.

mal position again, the relief stop-cock was closed and a tracing taken. With these precautions the tambour was under an equal atmospheric pressure inside and outside while registering the movements. If the relief stop-cock was closed before all the surplus air had escaped, the lungs did not thoroughly collapse and the tambour registered some movements, although the thorax was open and the attachments of the heart thoroughly removed; the movements disappeared as

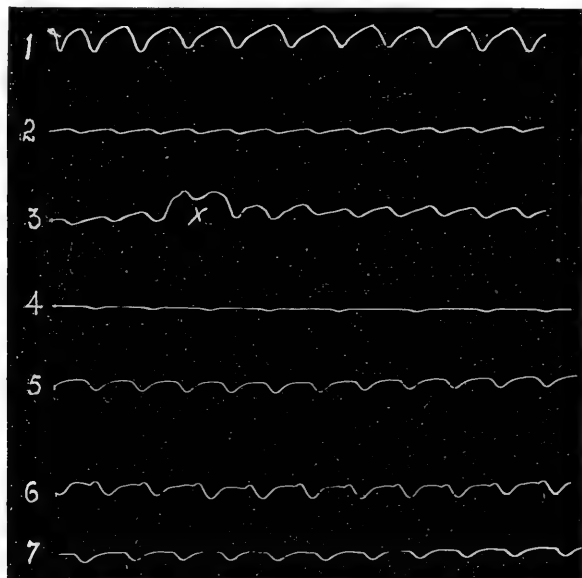


FIG. 4. (1) Shows the cardiopneumatic movements in a small dog with chest unopened. (2) Taken after the left pleural cavity was opened, the undulations becoming very small. (3) The continuation of tracing 2; closing the opening with a towel caused the movements to increase again. (4) Taken after both pleural cavities were opened; there is only a trace of the movements to be noticed. (5) Taken under the same conditions, but after the attachments of the heart to the front wall and the diaphragm had been thoroughly removed. The movements are again considerably increased, but they differ in character from the normal pneumocardiograms of the same dog, and resemble tracing 2 in Fig. 1 (from Haycraft and Edie, see above). (6) Taken under the conditions of tracing 5, but both openings were closed with towels. The size and especially the character is changed. (7) The towels were removed and the tracings are once more the same as in 5.

soon as the air was permitted to escape thoroughly. These movements were quite large at times, especially when the attachments of the heart were thoroughly removed. The tracing in Fig. 5 illustrates this case.

While this tracing was being taken, both pleural cavities of the dog were wide open and the attachments of the heart removed, but the lungs were still considerably inflated and the heart was buried between them. The artificial respiration was interrupted for a long period; the dyspnoea caused a slowing of the beats of the heart, which, becoming quite large during each diastole, pressed the lungs to the sides of the thorax, and hereby caused large but not characteristic cardiopneumograms. Haycraft and Edie, it should be remarked, do not tell us what precautions they have taken to avoid

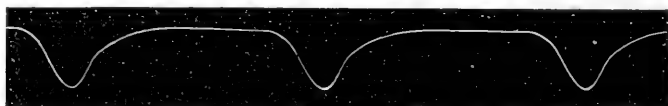


FIG. 5. Cardiopneumatic curve taken from a large dog. Dyspnoea; thorax wide open; attachments of heart thoroughly removed, but lung still quite inflated.

this artificial condition of affairs. We read there: "On stopping the respiration, opening the communication with the tambour, and clamping the tube passing to the bellows, the cardiopneumatic movements were well seen" (p. 430). I know by experience that even with an opening widely communicating with the air, it takes some time for an artificially ventilated lung to reach the limit of collapse. Haycraft and Edie do not tell us explicitly that they have taken the necessary precautions; perhaps the lungs of their animals still contained surplus air when the tracings were taken, giving them artificial cardiograms not to be obtained under normal conditions.

My experiments justify the following conclusions:—

(1) The cardiopneumatic movements disappear when both pleural cavities are open, provided the heart retains its normal attachments and the lungs are thoroughly collapsed before the tracings from the trachea are taken;

(2) The results of Haycraft and Edie might have been caused by their method of opening the thorax, which permits the falling back of the heart upon the lungs, and also might have been influenced by an incomplete collapse of the lungs;

(3) Without the assistance of the elasticity of the rubber cover of the tambour, the lungs in the open chest cannot follow the receding heart in the systole and no inspiratory movement can occur;

(4) The hypothesis offered by Haycraft and Edie that the lungs act like an "oncometer" placed around the heart cannot be accepted as an explanation of the normal cardiopneumatic movements.

For these reasons I agree with the majority of the writers that the cardiopneumatic movements have their origin in variations of the intrathoracic pressure caused by certain changes in the circulation.

# THE FUNCTIONS OF THE EAR AND THE LATERAL LINE IN FISHES.<sup>1</sup>

By FREDERIC S. LEE.

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ALL vertebrate morphologists are well aware that two of the prominent vexed problems of their science are the history, ontogenetic and phylogenetic, of the ear and that of the system of sense-organs usually called the sense-organs of the lateral line, a structure found in Fishes and aquatic Amphibia, larval or adult. Into the differences of opinion and confusion in this field of research Beard,<sup>2</sup> in 1884, introduced a semblance of order, and at the same time gave cause for much further contention, by bringing the two organs, or systems of organs, together phylogenetically, and claiming the ear to be nothing more nor less than a modified portion of the system of the lateral line. Since then much has been written upon this phase of the subject, the chief single contribution, perhaps, being that of Professor Ayers,<sup>3</sup> who adopted and developed very fully the general idea suggested by Beard.

The chief morphological facts upon which this view is based, are, in brief, the following:—

1. *Similarity in ontogeny.*—The ear and the lateral line seem to develop from a common ectodermal thickening, which extends forward and backward from the place of origin.

2. *Similarity in structure of adult organs.*—It is now generally acknowledged by morphologists that the internal ear is not one organ, but a group or system of organs, comprising in the fully developed ear of the fish seven recognizable patches of sensory epithe-

<sup>1</sup> The substance of this article was presented before the Section in Physiology of the British Association for the Advancement of Science at its meeting in Toronto, August 19, 1897.

<sup>2</sup> BEARD, J.: On the segmental sense-organs of the lateral line, and on the morphology of the vertebrate auditory organ. *Zool. Anzeiger*, 1884, vii, p. 140.

<sup>3</sup> AYERS, H.: Vertebrate cephalogenesis. II. A contribution to the morphology of the vertebrate ear, with a reconsideration of its functions. *Journal of morphology*, 1892, vi, p. 1.



lium: the three *cristæ acusticæ*, contained in the walls of the ampullæ of the semicircular canals; the three *maculæ acusticæ*, contained in the recessus utriculi, sacculus, and lagena; and the rudimentary *macula neglecta*, contained usually in the utriculus. The structure of these is well known; in each there is a localized area of sensory epithelium, supplied by special nerve-fibres and consisting of hair- and supporting-cells. The processes of the hair-cells project into a liquid, the endolymph. In addition to these parts, common to both *cristæ* and *maculæ*, each *macula* bears a mass of otoliths, resting upon the sensory hairs. The labyrinthine cavity of the internal ear in the Selachians is more or less freely open to the outside medium through the *ductus endolymphaticus*.

The sense-organs of the lateral line are numerous, and are either free or contained in canals lying in the skin of the head and the sides of the body. These canals are open at intervals directly to the water of the medium, as are the semicircular canals through the endolymphatic duct. From their position, different portions of the lateral system have received the names *supraorbital*, *infraorbital*, *mandibular*, *occipital*, and *lateral*. The structure of the sense-organs closely resembles that of the *cristæ acusticæ*: in each there is a localized area of sensory epithelium, supplied by special nerve-fibres and consisting of hair- and supporting-cells; the processes of the hair-cells project into a liquid which, when canals are absent, is the water of the medium, and, when canals are present, is this water plus more or less mucus excreted by the cells lining the walls of the canals.

3. *Similarity in innervation.* — The sense-organs of the lateral line are innervated by nerve-fibres forming well-marked bundles, which run to the periphery usually in company with branches of the fifth, seventh, ninth, and tenth cranial nerves. To speak of them as being innervated by these cranial nerves is misleading; the connection of their nerves with the cranial nerves is secondary. In reality, the nerves of the lateral line form a definite system of their own with a common central origin, which central origin is, likewise, the central origin of the eighth or acoustic nerve.

It must be acknowledged that these facts are of great importance, and seem to warrant the interpretation given to them.

Can physiology throw any light upon this question? A study of function is not usually believed to be capable of elucidating the genetic relationship of organs, and hence most writers have wholly neglected the physiological side. It might have been advantageous if

more had done this, for the physiology that has thus far been introduced is largely speculative, usually lacks an experimental basis, and is at times grotesque. What is needed here, as in all similar cases, is not speculation from morphological facts as to function and the origin of function, but reasoning from physiological facts that are first acquired by rigid and critical experiment. We have here an admirable opportunity to demonstrate not only that the phylogeny of function is worthy of study for its own sake, but that physiology is at times able to present, if not direct, at least valuable circumstantial evidence of the truth of morphological deductions. The evidence that I wish to bring forward has been obtained, unless otherwise stated, by myself from experiments upon fishes at the Marine Biological Laboratory, Woods Holl, Mass., during recent summers. The form chiefly used was the common smooth dog-fish (*Galeus canis*, Mitchill) of our Atlantic coast.

Some of the results have already been published, but must be mentioned briefly in this connection for the sake of rendering the subsequent work more easily understood. No attempt will be made to discuss the experiments of others upon higher animals, as this for my present purposes would be quite superfluous. The work is not yet completed.

We will consider the functions of the various sense-organs in order.

## I. THE EAR.

A. *The cristæ acusticæ*. — The experiments here have been clear-cut, and the results have appeared with a mathematical exactness rarely realized in physiological investigation.

One general function is here present, namely, the appreciation of rotary movements of the body, *i. e.*, movements in curved lines. Hence, the semicircular canals with their sense-organs are organs of equilibration.

The methods of arriving at this conclusion were three: turning the normal fish in the planes of the canals and other planes; stimulating separately and in combination the various ampullar branches of the acoustic nerve, which in the fish are separate and very easily reached; and cutting these ampullar branches. The index to the state of the animal's sense of equilibrium was found in the compensating movements of the eyes and the fins. The results obtained by these three methods are entirely harmonious. Thus, turning the normal

animal in a definite plane, either the plane of a canal or a plane between two canals, causes the eyes and fins to make certain definite and constant movements, which indicate a recognition of the body-movement and an attempt to resist or overcome it. If, now, the canals of the corresponding planes be exposed in another animal, and their cristæ stimulated artificially, exactly the same compensating movements of eyes and fins are made. The inferences are: first, that the operated fish has the sensation of being turned out of his normal position; and second, that when a normal fish turns in the planes specified, he stimulates the corresponding cristæ. This latter inference is corroborated by cutting the nerves supplying the cristæ in question, and then turning the animal in the same planes as before. This has not been done for single cristæ, and it is quite possible that in such case the others would maintain the compensation; but it has been performed for a functional pair of cristæ, and the result is absence of the normal compensating movements pertaining to the canals in question. The harmony of the results of these three methods is striking.

Each canal with its sense-organ has a principal and a subordinate function: the former is the appreciation of rotary body-movements in the plane of the canal and toward its side of the body; the latter is the appreciation of similar movements in the same plane but in the opposite direction. Each canal has a functional opposite, the two lying approximately in the same plane, and the principal function of one being the subordinate function of the other. Such functional opposites are the right anterior and the left posterior, the left anterior and the right posterior, and the right and left horizontal canals. A simple movement of the body in the plane of a canal puts the canal into most complete action; a movement in any other plane may be regarded (from the standpoint of the canals) as a compound movement, and may be resolved into simple movements in the planes of two or more canals. Such compound movements are appreciated by the two or more canals acting as a double organ (functional pair), or triple organ, which likewise possesses its principal and subordinate functions. The action of a functional pair, or double organ, is exemplified in the appreciation of diving movements by the two anterior canals, and in the appreciation of the movement of shooting upward in the water by the two posterior canals.<sup>1</sup>

<sup>1</sup> For a fuller discussion of the functions of the canals the reader is referred to my articles already published. Ueber den Gleichgewichtssinn. Centralbl. f.

B. *The maculæ acusticæ*. — Here not so much work has been done, and the results are not as sharp-cut as in the case of the *cristæ*. Artificial stimulation of the maculæ has been tried in a large number of cases, but does not appear to give consistent results. Cutting the macular nerves or, perhaps better, removing the otoliths that always cover the maculæ, makes a decided general change in the conduct of the animal, which to one familiar with the movements of fishes seems to indicate unmistakably the lack of certain sensory functions. It should be premised that, in the form studied chiefly, the utricular and saccular maculæ are very closely contiguous, and the macula of the lagena is simply a small continuation of that of the saccule; hence, so far, it has been impossible to separate the three functionally, and it is very questionable whether the division of labor here is as sharp-cut as with the *cristæ*.

Two functions seem to be present, both of which are equilibrating: the appreciation of translatory or progressive movements, or movements in straight lines, and the appreciation of the position of the body in space.

1. *Appreciation of progressive movements*.<sup>1</sup> — A normal fish has a delicate sense of the distance involved in swimming in a straight line. This is shown by the remarkable skill with which he avoids obstacles; in swimming around his aquarium constantly, he strikes his nose directly against the side of the tank comparatively rarely. This is not so with a fish deprived of all his otoliths or with all his macular nerves severed. Such a fish seems to have little idea of the extent of a forward swim. He is often restless and frequently alters the direction of his progression. In moving along close to the side of a large oblong tank he often swims at his usual rate and with his usual freedom, and appears fairly normal; but, upon reaching the end, it frequently happens that he does not turn, like the normal fish, gracefully to one side and thus avoid the end-wall. On the contrary, he often bumps directly into it. When excited by a stick, he darts about in different directions, irregularly and with awkward,

Physiol., 1892, vi, p. 508. A study of the sense of equilibrium in fishes. Part I. The Journal of physiology, 1893, xv, p. 311; and Part II, *ibid.*, 1894, xvii, p. 192.

<sup>1</sup> A full account of my observations upon this function and upon the subject of hearing in Fishes has not been published. My observations were communicated to The American Physiological Society, 1894, Dec. 28; a brief abstract of that Paper was printed in "Science," 1895, Feb. 1, N. S. i, p. 118, and a still briefer one in the Centralblatt für Physiologie, 1895, April 6, ix, p. 47.

uncertain, and ill-regulated movements, often shooting far out of the water. Moreover, after swimming into a corner, his movements are awkward and he finds great difficulty in retreating or getting back into open water. If he succeeds in doing this, he may return at once to the same corner and repeat his ungainly attempts at extricating himself. It is easy to draw a hasty inference that the eyesight of such a fish must in some mysterious way be at fault. As a matter of fact, however, a fish deprived of his eyesight, but in other respects normal, does not conduct himself in that way. When left to himself, the blinded fish<sup>1</sup> swims normally in all respects, moving gracefully, easily, and without timidity, and shooting and diving like an uninjured fish. When disturbed by being poked he darts about with great rapidity, but is master of his movements. In the first few hours of his blindness he bumps the end of the tank in progressive swimming, but soon he largely overcomes this, and, probably by his sense of touch, learns to avoid gross contact with such obstacles. He then touches the walls lightly, glides away from them easily, and only when disturbed bumps as at first. When by chance he swims into a corner, he extricates himself easily, gracefully, and with certainty. The compensating movements of his eyes and fins are entirely normal.

It is thus seen that the fish deprived of retinal sensations and the fish deprived of macular sensations act very differently. The conclusion that in the latter animal the appreciation of progressive movements is dulled or largely eliminated seems quite sufficient to explain the phenomena.

We thus find experimental proof — and the first thus far reported in animals lower than man, if I am not mistaken — of the presence in the otolithic parts of the ear of a power to appreciate progressive movements. That such a function must exist there was ably advocated by Breuer,<sup>2</sup> who gave reasons for the probability "that the perception of rectilinear movements is sharper in Fishes and Birds than we find it in observations on ourselves."

2. *Appreciation of position in space.* — The fact that a normal fish has a delicate sense of his position in space, of upness and downness, is demonstrated by the difficulty experienced in inducing one to rest

<sup>1</sup> In blinding, the contents of the eyeballs, including the retinas, were removed; the sclerotic coats with their muscular attachments were left *in situ*.

<sup>2</sup> BREUER, J.: Ueber die Function der Otolithen-Apparate. Arch. f. d. ges. Physiol., 1890, xlviii, p. 195.

in any other position than that with the back upward and the belly downward. He not only resists with all his powers being turned over (this movement is appreciated by the semicircular canals), but with equal energy he resists being compelled to remain out of his customary position; and, when put out of the latter and then left to himself, he at once assumes his normal attitude.

It is not so with a fish from which the otoliths have been removed, or in which the macular nerves have been cut. If either of these operations be done upon one side only, the eyes and fins are placed in abnormal positions; in reclining, the operated side is usually slightly downward; normal swimming is possible, but here usually the operated side is depressed; when disturbed, the fish usually darts off with that side downward; and in coming to rest he often loses his balance and sinks down flat upon that side, never upon the other. If the operation be performed on both sides, the fish seems to have largely lost his sense of upness and downness. While preferring usually the normal attitude, it is not difficult to make him remain lying on his back or side; and not rarely he is found in such abnormal positions, having assumed them himself. He swims at times upon his side, and occasionally upon his back. He seems not to appreciate at all clearly his position in space.<sup>1</sup>

I have found no reason to disbelieve that the same otolithic parts of the ear appreciate both progressive movements and position in space, which is not in accordance with the doctrine of specific energy in its extreme form. The view of Breuer,<sup>2</sup> however, seems wholly reasonable, namely, that changes in the pressure of the otoliths are appreciated as changes of position in space when they are accompanied by sensations (ampullar) of rotary movements, and as progressive movements when not so accompanied.

It is evident that there is a close functional connection, through the central nervous system, between the sensory organs of the ear, the cristæ and maculæ, and the locomotor organs of the body. A reflex arc with the evident function of equilibration is thus established. The idea that the cristæ and maculæ are sensory organs of this function does not preclude acceptance of the well-established fact that other

<sup>1</sup> For a fuller discussion of this statical function of the maculæ, the reader is referred to the author's articles in the *Centralblatt für Physiologie*, 1892, vi, p. 508, and the *Journal of physiology*, 1893, xv, p. 311.

<sup>2</sup> BREUER, *loc. cit.*

sensory organs, such as the eyes, are similarly connected by reflex arcs with the locomotor organs, and similarly functionate as sensory organs of equilibration. In these latter organs equilibration is accessory to other chief functions, as in the eyes to sight; in the cristæ and maculæ it is the chief, and perhaps the only, function.

The functions of the ear of the fish so far considered may be put in convenient tabular form as follows: —

I. Dynamical functions, in recognition of	{	1. Rotary movements, mediated by cristæ acusticæ.
		2. Translatory movements, mediated by maculæ acusticæ.
II. Statical functions, in recognition of	{	3. Position in space, mediated by maculæ acusticæ.

As to the manner of stimulation, it is evident that both the cristæ and the maculæ belong to the category of pressure-organs. This is most evident in the maculæ, in which the pressure of the otolithic mass upon the delicate sensory hairs affords a constant stimulus which varies in intensity with the position of the animal in space, or with the force of the progressive movement.

In the cristæ, the most reasonable mode of stimulation appears to me to be that due to the inertia of the endolymph as the hair-cells are moved through space along with the body. The result is a greater pressure upon one side of the hairs than upon the other, and hence a stimulation of the sensory cells by pressure.<sup>1</sup>

<sup>1</sup> In a recent article E. v. Cyon (Bogengänge und Raumsinn. Experimentelle und kritische Untersuchung. Archiv für Physiologie, 1897, p. 29) appears in the light of a sceptic regarding the results obtained in my experiments on dog-fishes, apparently because of their exactness and completeness. Among other things he reproaches me with the fact that "Lee hat . . . immer alles bestätigt gefunden, was die Hypothese erheischt." This fact will occasion no surprise, when it is borne in mind that the great majority of my results were obtained long before the "hypothesis" as formulated by myself was made (cf. LEE: Journ. of physiol., 1893, xv, p. 321), and long before I was acquainted with the work of Breuer. The theory as formulated in my chief Paper was a direct induction from the facts obtained by myself; I took pains to keep it within the limits of those facts, and thus it was that I "always found what the hypothesis demanded." In a subsequent Paper (LEE: *ibid.*, 1894, xvii, p. 192) I aimed "to fill in certain gaps that were left in the previous series, to make the proof of certain points more logically complete, and experimentally to verify certain inferences and prophecies resulting from the theory formerly stated." As regards the exactness of my results, I have often felt and expressed surprise similar to that which Cyon apparently feels. Yet I have endeavored accurately to chronicle the phenomena

C. **The question of hearing.** — We thus appear to have found a function for all of the sensory organs of the ear of the fish. But the question naturally arises — does not the fish possess audition? In higher vertebrates, of course, this is present, and we can add to the above scheme:

III. Auditory functions, in recognition of { 4. Vibratory motions, mediated by papilla acustica basilaris.

This exhausts all possible cases of mechanical (*i. e.* visible) motions in matter. It is interesting that the ear is the sense-organ that appreciates all kinds of visible motion.

Wherever among vertebrates undoubted audition exists, there is present an additional group of sensory end-organs, the papilla acustica basilaris. This does not exist in Fishes, but appears first in the Amphibia as an offshoot from the lagena, and in higher vertebrates constitutes the nervous portion of the organ of Corti of the cochlea.

actually observed under the circumstances of the many and widely varied experiments.

Cyon further seems to regard the integrity of the sense of sight as necessary to the performance of the compensating movements of the eyes, and makes the astonishing statement that “. . . der Dogfish (*Galea canis*) tagesblind ist, wie Beer unzweifelhaft bewiesen hat.” On the contrary, Beer (*Die Accommodation des Fisches*, Arch. f. d. ges. Physiol., 1894, lviii, p. 577) has neither stated nor attempted to prove such a thing. He says: “Wir haben einigen Grund anzunehmen, dass die Pupille der in Freiheit lebenden Fische wegen der relativen Dunkelheit grösserer Tiefen noch etwas weiter sein wird, als bei den Thieren in den gewöhnlichen Versuchsbassins, die in einer für sie vielleicht blendenden Helle leben. Bei vielen Haifischen und Rochen tritt sogar in den dunkleren grossen Aquarien der Station tagsüber eine so starke Miosis ein, dass die Pupille — die Nachts, resp. im Dunkeln sehr weit ist und dann auch hier den Linsenrand sehen lässt — fast gar nicht sichtbar, sondern . . . verschlossen wird; die Thiere benehmen sich auch wie blind,” etc. As a matter of fact, under the circumstances of my experiments, the pupils of *Galeus canis* varied greatly in size, from being wide open to being partially closed. But I do not now recall a single instance of their being wholly closed, nor have I ever seen an uninjured individual of this species behaving during the daytime like a blind fish. In an experience of three summers this species has not exhibited himself as “blind in daylight.” This question is, however, of minor importance, since, as stated above, the eyeballs from which the contents, including the retinas, are removed, perform the normal compensating movements. Cyon appears not to comprehend the fact accented above, namely, that the movements of the eyes are reflex movements, the afferent stimulus to which comes from the *cristæ acusticæ*. This does not preclude the further undoubted fact that under normal circumstances the movements are accompanied by sensations of sight.



At first sight the presence of the "ear" in Fishes presupposes a sense of hearing; but the absence of the papilla acustica basilaris argues against it. The fact that fishes, with comparatively few exceptions, are dumb seems to me strong evidence against the possession by them of a sense of audition. It would seem that the primary purpose of the power of emitting sound by an organism is communication with other individuals of its own species. Similarly, the primary purpose of a sense of hearing is apparently the gaining of information regarding the presence of other individuals of one's own species. In each case the employment of the function for other purposes would be secondary. If this be so, we should expect the two powers of emitting sound and of hearing sound to be developed in the same individuals and developed more or less *pari passu*. A survey of the various groups of animals shows this to be the case. Further, it is difficult to conceive upon any grounds the object or usefulness of the possession by Fishes of the power to hear in the sense in which the term is ordinarily used. The depths of the sea are silent, the sounds of the waves probably penetrate only the most superficial layers of the water, and aquatic animals, with comparatively few exceptions, are dumb. Along the shores some outside sounds might penetrate the water superficially, but such would form only a very slight exception to the general law that in nature water, unlike air, is devoid of sound.

But experimentation can best answer the question as to the power of audition in Fishes.

In the summer of 1894 I tested a number of species as to their power of hearing, employing ordinary sounds, such as the human voice, clapping the hands, and striking stones together in the air and under water.<sup>1</sup> I obtained no evidence whatever of the existence of a sense of hearing as the term is customarily employed, although I learned that fishes are exceedingly sensitive to gross shocks, such as the jarring of their tank or concussions upon its walls, *i. e.*, such vibrations as human beings recognize through other sensory mechanisms than the ear as distinct mechanical vibrations. The accompaniment of such apparently gross vibrations was the indispensable condition upon which the fishes in my experiments reacted to sounds. I have learned recently of Bateson's similar experiments<sup>2</sup> which were

<sup>1</sup> See footnote p. 132.

<sup>2</sup> BATESON, W.: The sense-organs and perceptions of fishes. *Journal of the marine biological association*, 1890, i, p. 225.

interpreted in much the same way. In 1895 Kreidl<sup>1</sup> published the results of a considerable series of experiments carefully conducted upon goldfish. He used as sources of sound in the air various whistles, electric and other bells, clapping of the hands, and the firing of a revolver, and in the water vibrating rods; and he studied their effects upon normal fishes, those whose excitability was greatly increased by strychnine, and those that had been deprived of their ears. Neither normal nor strychnized fishes showed any reaction whatever to musical sounds of any pitch or intensity, produced in the air or water. Concussions, such as striking the walls of the aquarium in the case of normal animals, or clapping the hands or firing a revolver in the case of strychnized animals, produced marked reactions, especially in the latter; but such reactions were equally well-marked after both ears were removed, and still remained after the removal of the whole cerebrum and a portion of the mid-brain. His experiments leave no doubt of the correctness of his conclusions, which were: "(1) That hearing through the 'auditory organ' cannot be demonstrated in the goldfish; (2) nevertheless, they react to sound waves, which they perceive through a specially developed skin-sense."

In a subsequent paper Kreidl<sup>2</sup> explodes the oft-repeated tale of hearing by fishes that come for their food at the sound of a bell, by investigating carefully the actions of trout at the famous old Benedictine monastery in Krems, Austria. He proved that the fishes come because they see the man who brings the food, and appreciate the vibrations of the water caused by his step and communicated through the stone basin; and that, when these are excluded, the sounds of the bell have no effect.

The conclusion seems justified beyond doubt that fishes do not possess the power of hearing, in the sense in which the term is ordinarily used.

We must believe that in vertebrates this sense was evolved along with a change of the mode of life from a water to a land existence, and was contemporaneous with the appearance of a papilla acustica basilaris.

It appears plain that the sole function of the ear in Fishes is equilibration.

<sup>1</sup> KREIDL, A.: Ueber die Schallperception der Fische. Arch. f. d. ges. Physiol., 1895, lxi, p. 450.

<sup>2</sup> KREIDL, A.: Ein weiterer Versuch über das angebliche Hören eines Glockenzeichens durch die Fische. Arch. f. d. ges. Physiol., 1896, lxiii, p. 581.

The mechanism by which Fishes appreciate certain mechanical vibrations and which Kreidl proved not to reside in the ear, is no more understood in them than it is in other animals and in human beings. This power is wide-spread in animals, even in many Invertebrates in which there is an absence of the power of hearing, *e. g.*, in earthworms, and it is astonishingly developed in some human beings. My friend, Professor Hallock, tells me that Helen Keller, who, it is well known, is totally deaf and devoid of other special senses except that of touch, is able, by keeping her fingers in contact with the larynx of a person who is singing, to appreciate accurately the changes in pitch; she can thus accurately reproduce with her own voice the notes sung by her companion. There is no question here of the absence of the sense of hearing, yet the vibrations are appreciated. As regards these two sense-activities, the case of the normal fish is without doubt analogous to the case of such defective human beings.

## II. THE LATERAL LINE.

The original view of the function of the lateral line was that its canal produced mucus for moistening the surface of the body of the fish. When in 1850 Leydig<sup>1</sup> discovered the numerous sense-organs in it and later ascribed to them the mediation of a sixth sense, the original view was laid aside for others of a sensory function. Upon little experimental evidence it has been suggested that the organs are true touch-organs, are auditory organs, that they appreciate currents in the surrounding water, the chemical nature of the water, and so on. There has been no consensus of opinion as to their exact function or mode of action.

During the summers of 1892, 1893, and 1894, I carried on at different times experiments of various kinds upon the lateral-line system (not including the ampullæ of Lorenzini or Savi's vesicles). I studied several species of fishes, especially the dog-fish (*Galeus canis*, Mitchill), the toad-fish (*Batrachus tau*, L.), and the butter-fish (*Stromateus triacanthus*, Peck), and obtained certain suggestive results. The main points will be communicated here.

In the dog-fish the lateral-line organs of the head are supplied by the superficial ophthalmic, the buccal, and the hyomandibular branches of the seventh nerve; those of the body by the so-called lateral branch of the vagus. In this species I have cut all of these

<sup>1</sup> LEYDIG, F.: Ueber die Schleimkanäle der Knochenfische. Müller's Arch., 1850, p. 170.

branches, and, moreover, have stimulated the central end of the lateral branch. In the toad-fish, where the organs are normally exposed and not enclosed in canals, I have destroyed by thermocautery all of the organs upon one and upon both sides of the body. In general, it may be stated that the results indicate that the organs of the lateral line have a sensory function, closely connected with the motor organs and analogous to the functions of the ear, and hence may be regarded as organs of equilibrium.

Simple cutting of the lateral nerve on one side or even both sides, cutting of all the nerves supplying the lateral-line organs of both head and body, or destruction of all the organs themselves, does not seem to interfere much, if any, with the animal's equilibrium. I have observed at times an abnormally slow return to the normal position after an animal so operated upon has been turned upon his back, but whether this was due to dulling of a sense of equilibrium or to general weakness, I am unable to say. All these experiments were performed more than five years ago, however, and before experience had taught me to observe and interpret interferences with equilibrium, hence I would not now be willing to make a categorical statement regarding the effects of operations of this kind. The animals so operated upon are always much less active than before; they swim little, lie quiet, and rarely survive more than a day or two at most.

An extension of such operations has given more decided results. It occurred to me that a reduction of the motor mechanism employed in maintaining equilibrium might be an indirect aid in demonstrating a possible equilibrium function in the lateral-line organs. In the toad-fish, which spends much of its time lying upon the bottom of the sea, especially in holes under stones, the pectoral fins are enormously developed and seem to act as mechanical supports. Removal of these fins does not, however, of itself cause marked inconvenience in the maintenance of statical equilibrium or the performance of movements; the subsequent removal of the two smaller ventral fins is followed by no apparent genuine lack of the power to appreciate equilibrium and only a comparatively slight handicapping of the power of movement. It is remarkable how closely the actions of a fish deprived of these four fins resemble those of a normal fish; he is lively, vicious, quick in responding to mechanical stimuli, and certain in his movements; he guides himself accurately, turns about suddenly, and moves forward without lateral swaying of his body. But removal of the four fins combined with destruction of all the

organs of the lateral line (except the supraorbital ones, which are few in number and lie too deep for ready operation), produces a marked effect. This has been performed in several individuals. No genuine forced movements seem to follow, but there are decided evidences of a lack of the sense of equilibrium. This is manifested in various ways. There is decided uncertainty in movement, the animal swaying from side to side in forward progression. Such a fish is easily turned upon his side or back and lies quiet in this position. In one case, some two hours after the operation, the toad-fish was turned upon his back upon the bottom of his aquarium and remained thus for fifteen minutes, apparently without discomfort, and without attempting to return to the customary position. He was then returned to the latter by the hand. Such a fish swims irregularly upon his back or his side, with a general preference for the normal attitude. In one case in which the lateral organs of one side only were destroyed, and the pectoral and pelvic fins were removed, the power of maintaining equilibrium was found to be markedly weakened. When tested five hours after the operation, and presumably after the direct shock of it had passed away, the fish lay on his back when placed there; in swimming upward he thrust his head far out of water in much the same way as a fish deprived of his otolithic organs; and he showed the lateral swaying movements and uncertainty in forward progression, mentioned above. On the next day he exhibited the same phenomena. All the actions of a fish so operated upon remind one much of a lobster that has lost his otolithic sacs and chelæ.<sup>1</sup> The phenomena indicate strongly that the organs of the lateral line have something to do with equilibrium. In this connection it is of interest to know that Bonnier<sup>2</sup> has recently found that fishes whose lateral organs have been destroyed by galvano-cautery have largely lost the capacity of correct orientation in presence of disturbances in water.

It might be objected that the severity of the operation of cauterizing so many spots in the skin and removing the four fins might of itself be the cause of the disturbances of equilibrium above described. I have anticipated and nullified the possible force of such an objection by cauterizing an equal number of spots upon the skin of a nor-

<sup>1</sup> BUNTING, M.: Ueber die Bedeutung der Otolithenorgane für die geotropischen Function von *Astacus fluviatilis*. Arch. f. d. ges. Physiol., 1893, liv, p. 531.

<sup>2</sup> BONNIER, P.: Sur le sens lateral. Comptes rendus de la soc. de biol., Paris, 1896, p. 917.

mal fish, carefully avoiding the lateral line, and then removing the four fins. Such an animal does not behave differently from one lacking the fins and not otherwise injured; he is active and certain in his movements, shows no lack of equilibrium, and in general closely resembles a normal fish.

But perhaps more significant than the above experiments on elimination is the result of stimulating the central end of the lateral nerve. In the dog-fish I have cut the nerve upon one side and stimulated it by an induced current just behind the head, thus including the nervous supply of all the organs of that side of the trunk. The result is perfectly coördinated, definite movements of the fins of both sides of the body. These fin-movements are the same as those called out by stimulating centrally the cut acoustic of the opposite side, and the same as those resulting from section of the acoustic of the same side. Thus, stimulating the left lateral nerve centrally caused: the two dorsal fins to move to the right; the caudal fin to move to the right; the left pectoral fin to move upward; the right pectoral fin to move downward; the two pelvic fins to move like the corresponding pectorals. This is exactly the result of stimulating the central stump of the right acoustic,<sup>1</sup> and also the exact result of cutting the left acoustic.<sup>2</sup> In the case of the acoustic I have shown that the result of stimulation is only what would be expected from a simultaneous and equal stimulation of the three ampullar branches of the acoustic. Since these branches are plainly equilibrative in function, it would seem to suggest strongly that the lateral nerve is also equilibrative. Occasionally, movements of the fins in directions opposite to those specified above take place, but they are by no means so constant or persistent, and, thus far, I have not attempted to determine the conditions of their appearance. In this connection it may be recalled that moderate or strong stimulation of the ampullæ of the semicircular canals causes movements of the eyes in a certain direction (principal function), and slight stimulation causes movements in exactly the opposite direction (subordinate function).<sup>1</sup> Movements of the trunk also result from stimulation of the lateral nerve, but thus far I have not endeavored to analyze them.

This is the extent of the experimenting that I have performed upon the lateral line, but, so far as it goes, it seems very suggestive. It would be interesting to test the lateral nerve at different parts of its course, and observe whether movements of the fins of different

<sup>1</sup> LEE: Journ. of physiol., 1894, xvii, p. 192.

<sup>2</sup> *Ibid*, 1893, xv, p. 311.

segments result. Further, I have observed no eye-movements from lateral stimulation. It is conceivable that eye-movements may be mediated by the lateral-line organs of the head only, but so far I have not tested this possibility by stimulating the branches of the seventh nerve.

Without further work, which is already projected, it is impossible to specify in detail the nature of this possible equilibrative function of the sense-organs of the lateral line. The phenomena suggest Schulze's<sup>1</sup> idea of the possibility of appreciation of mass-movements of the water or of movements of the body through the water, just as the movements of the hairs of the cristæ through the inert endolymph mediate the appreciation of rotary movements of the body. Such a function seems more easily understood in the case of those fishes, such as the toad-fish, in which the sense-organs project freely to the outside, than in those in which the organs are enclosed within canals that open to the outside only at intervals. Yet in all species such a function seems to me *a priori* the most probable of all that have been suggested. The appreciation of rotary movements and that of progressive movements can hardly be separated here. It is not inconceivable nor improbable that, in addition to this function, contact with solid objects, such as the sand and stones at the bottom of the sea, may stimulate the organs and assist crudely in giving the fish a notion of his position in space. The structure of the organs suggests, as in the sense-organs of the ear, that they are pressure-organs of some kind. In this connection the work of Fuchs<sup>2</sup> is interesting. This investigator has shown by experiments upon *Raja clavata* and *Raja asterias* that touching the skin over the ventral canals of the head causes in most cases a negative variation of the current of rest in the cut nerves supplying these canals. He infers, not wholly with justification it seems to me, that the canals mediate the appreciation of changes of hydrostatic pressure.

In whatever specific way future experiments may prove the sense-organs in question to act, it is at least clear that those of the trunk are connected through the central nervous system with the muscles of the fins, and that a reflex arc is thus present, analogous in nature to that formed by the same motor mechanism with the acoustic nerve.

<sup>1</sup> SCHULZE, F. E.: Ueber die Sinnesorgane der Seitenlinie bei Fischen und Amphibien. Arch. f. mikr. Anat., 1870, vi, p. 62.

<sup>2</sup> FUCHS, S.: Ueber der unter der Haut liegenden Canalsysteme bei den Selachiern. Arch. f. d. ges. Physiol., 1895, lix, p. 454.

We must believe, moreover, that the impulses of whatever kind that come to the central nervous system from the organs in question assist in enabling the animal to maintain the equilibrium of his body: in other words, the organs of the lateral line are equilibrating organs.

Hence, a consideration of the physiology of the ear and the system of the lateral line, by showing that the two systems of organs are functionally analogous, would seem to offer indirect support to the morphological deduction that the former organ is a derivative of the latter. The primitive function, not improbably, was the appreciation of movements of the water against the body and movements of the body in the water, combined with appreciation of contact, and, hence, indirectly and crudely, of position in space; by the exercise of this function, through functional connection with the locomotor mechanism, the equilibrium of the body was maintained. In some unknown way a bit of this sensory system became cut off from the rest and enclosed within the skull; it still retained its power of appreciating bodily movements and contact, and this power became refined and differentiated; the capacity of appreciating rotary movements was separated from that dealing with progressive movements and position in space, and the two were associated with distinct organs, the semi-circular canals on the one hand, and the otolithic organs on the other, which were appropriately constructed to subserve their respective functions. Thus a well-marked sensory organ for equilibrium was evolved in fishes. When aquatic animals began to leave the water and live for a shorter or longer time upon the land, and the possible advantage of a sense of hearing was presented, a portion of this sensory organ of movement became still farther differentiated; a new patch of sensory nerve-terminations appeared, the papilla acustica basilaris; apparatus for conveying the waves in the air directly to the membranous ear was developed; and thus the power of appreciating the movements that we call sound was acquired. By natural selection this was still more refined and specialized, the range of appreciation was extended, and the result is the mammalian cochlea with its great functional powers.

It seems to me that this explains naturally and in a manner not improbable the mysterious association in one organ of two functions at first sight so widely separated as equilibration and audition.



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THE INFLUENCE OF THE HEART-BEAT ON THE  
FLOW OF BLOOD THROUGH THE WALLS  
OF THE HEART.<sup>1</sup>

By W. T. PORTER.

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IN 1689 J. Baptista Scaramucci<sup>2</sup> published two hypotheses which have played a great part in the history of the physiology of the heart: the first, that the deeper coronary vessels are squeezed empty by the contraction of the muscle fibres which surround them; and the second, that the coronary vessels are refilled from the aorta during the diastole of the heart. In 1707, Stroem<sup>3</sup> added to these the hypothesis that the coronary vessels are filled in diastole because their mouths are closed in systole by the semilunar valves. Thus, almost at the outset, the penetrating conception of Scaramucci was linked with an obstinate and overshadowing error, destined to be a source of controversy for nearly two hundred years.<sup>4</sup> Thebesius,

<sup>1</sup> The first account of my observations on the compression of the intramural vessels by the systole of the heart was published in the Journal of the Boston Society of Medical Sciences, No. 10, March 30, 1897. Most of the facts have been presented also to the American Physiological Society, in Washington, May 5, 1897, and to the British Association for the Advancement of Science, Toronto, 1897.

<sup>2</sup> SCARAMUCCI: *Diario Parmense*, 1689. Quoted by HALLER: *Element. physiolog.*, 1778, lib. iv, s. v, p. 459.

<sup>3</sup> STROEM: *Nova theoria machinæ animalis*. Quoted by HALLER: *loc. cit.*

<sup>4</sup> A sufficient account of the literature of this subject has already been given by CERADINI, G.: *Il meccanismo delle valvole semilunari del cuore*. Milan, 1871; *Der Mechanismus der halbmondförmigen Herzklappen*. Leipzig, 1872. See also REBATEL, F.: *Recherches expérimentales sur la circulation dans les artères coronaires*. Paris, 1872.

Vieussens, Morgagni, Boerhaave, and other famous eighteenth century men took sides for and against the theory of Stroem, and not until the century had passed, and many observers of the first rank had shown that the mouths of the coronary arteries are often beyond the reach of the semilunar valves, and that the pulse in these vessels is synchronous with the pulse in the aorta, did opinion come to rest on the filling of the coronary arteries in both systole and diastole. The calm that followed was brief indeed. About 1840, Marshall Hall attempted to revive the old belief, but was answered by the experiments of Kleefeld. Five years after Kleefeld, the controversy broke out afresh. Brücke on the one side and Hyrtl on the other, neither knowing that he was repeating arguments and observations that already filled many pages of cardiac literature, fought over the old ground, drew many with them into an extended and often unprofitable discussion, — and reached the old conclusion. Once more physiological opinion settled to the belief that the coronary arteries are filled during systole as well as diastole, — a position since rendered impregnable by the observations of Ceradini and the experiments of Rebatel and of Martin and Sedgwick.

Throughout this long discussion, the primary hypothesis of Scarabucci, namely, that the deeper coronary vessels are emptied by the squeeze of the fibres contracting around them, received but scant attention. Thebesius,<sup>1</sup> in the celebrated inaugural dissertation in which he gave the first accurate description of the cardiac veins that bear his name, had said that in “no way could the arterial blood be forced into the vessels of the heart, unless during diastole; because in systole, the contraction of the fibres is so intense that all blood would be forced out, from the arteries no less than from the veins, — a condition that actually can be observed in the hearts of amphibia — frogs and others — which appear all white and bloodless when contracted, but are red and swollen with blood when relaxed in diastole;” and such reasoning was accepted by many who forgot that the heart of the frog is almost wholly wanting in bloodvessels, and that the red color of the full ventricle is due to the blood which fills the ventricle, seen through its translucent walls.

After the middle of the present century, experiment grew bolder and speculation began to yield place to direct observation. Hyrtl,<sup>2</sup> in 1855, trying to prove that the coronary arteries were filled in both

<sup>1</sup> THEBESIUS: *De circulo sanguinis in corde*. Leiden, 1708, p. 14.

<sup>2</sup> HYRTL: *Ueber die Selbststeuerung des Herzens*. Vienna, 1855, p. 9.

systole and diastole, severed a coronary artery in the living rabbit, cat, and dog, and declared positively that only the upper segment spurted in systole, — a statement confirmed by Perls.<sup>1</sup> In 1876, Klug<sup>2</sup> drew a ligature about the rabbit's heart at the auriculo-ventricular junction while the heart was in full systole, and again while in diastole. He then coagulated the blood in the cardiac vessels by holding the organ some time in dilute sulphuric acid, and compared thin sections of the ventricle with regard to the amount of blood in their walls. The vessels of the heart ligated during diastole were filled with blood, while those of the heart ligated during systole contained little blood. But neither of these experiments can be said to be of value in our present inquiry: for the observation of Hyrtl, though accurate for his purpose, which was to determine which limb of the severed artery "spurted," is otherwise incorrect; and the method of Klug is open to objections based upon facts discovered since his time.

With regard to Hyrtl's work, it is true that the distal segment of a severed coronary artery does not "spurt," but it is also true, as will be shown in detail in the description of the writer's experiments, that blood is forced out of the distal segment with each contraction of the ventricle. The quantity which thus escapes is extremely small, but this is because the amount of blood contained in the distal segment of a severed "terminal" artery is always necessarily small. The anastomosis with neighboring vessels is too slight to permit of collateral circulation, and only a free collateral circulation can cause the distal end of a severed artery to bleed profusely.

Turning to Klug's experiment, let us consider first the heart ligated in systole. Klug slowed the heart in order to be sure of the moment of ligation. The reader will not need to be reminded that when the beat of the mammalian heart is considerably slowed by exhaustion, or by artificial means, as in Klug's method, the cavity of the ventricle is seldom, if ever, fully emptied. The observations of Pratt<sup>3</sup> have shown the ease with which the veins in the heart wall are filled from the cavity of the ventricle through the vessels of Thebesius. It is clear that even if a ligature could be drawn tight around the auriculo-ventricular junction in the precise fraction of a second during which the mammalian heart remains fully contracted, the relaxation of the

<sup>1</sup> PERLS, M. : *Archiv für pathologische Anatomie*, 1867, xxxix, p. 189.

<sup>2</sup> KLUG, F. : *Centralblatt für die medicinische Wissenschaften*, 1876, p. 134.

<sup>3</sup> PRATT, F. H. : The nutrition of the heart through the vessels of Thebesius and the coronary veins. *American journal of physiology*, 1898, i, p. 86.

ventricle after the tying of the ligature would fill its capillaries from the ventricular cavity, so that the amount of blood in the ventricular walls when the heart came to be examined would in no wise correspond to the amount present in the walls at the height of their contraction. If the heart is permitted to beat at its usual rapid rate, the ventricular cavities may be fully emptied at each stroke; but the time for the tying of the ligature is then so short that it is obviously impossible to be sure whether the ligation is made in full systole or a little before or after systole. If it were possible to be sure of the moment of ligation, and to make certain that the ventricular cavities were empty at that moment, and that the ligature shut off the auricles entirely, — the mural capillaries could still be filled when the heart relaxes from the blood in the large superficial coronary vessels, which are not within the grasp of the contracting fibres and cannot be compressed by them. In the heart ligated in diastole, it cannot be determined whether the blood found in the intramural vessels was present there at the moment of ligation, or entered the walls afterward through the veins of Thebesius or the superficial coronary vessels. Finally, the plunging of the fresh heart, warm from the body, into a coagulating bath of sulphuric acid, may so change the tonus of the ventricle as to alter materially the amount of blood in its capillaries. These sources of error render the observations of Klug unavailable.

It is Rebatal<sup>1</sup> whom we must thank for the first fruitful experiment in this field. Chauveau had given him the circulation in the coronary arteries as the subject of his inaugural dissertation, and had suggested that a **T**-tube should be placed in the right coronary artery of the horse and connected with an hæmodromograph, which should write a curve of the quickness of flow in the coronary artery, while at the same time a curve of the tension in the aorta should be recorded for purposes of comparison. Rebatal secured these curves, and saw at a glance that the beginnings of the upstrokes in the aortic and the coronary curves coincided exactly, showing that the blood-wave is synchronous in the two arteries, and that the coronary arteries are filled during systole. He saw also that the primary increase in the rapidity of current in the coronary artery was followed by a second augmentation "corresponding exactly to the moment when the aortic tension is least, *i. e.*, to the diastole of the heart. The first augmen-

<sup>1</sup> REBATEL, F.: Recherches expérimentales sur la circulation dans les artères coronaires. Paris, 1872.

tation," Rebatel asserts, "is evidently due to the propulsion imparted to the column of liquid by the contraction of the ventricle; the second current may be due to the entrance of a new wave from the aorta

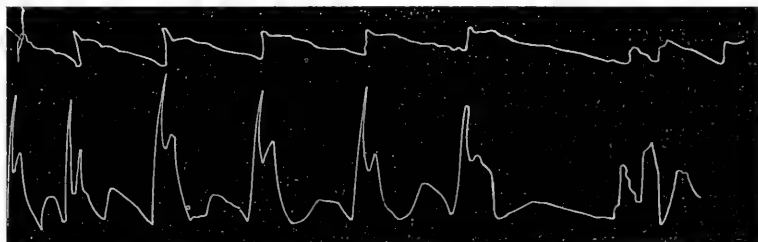


FIG. 1. Curves of the tension in the aorta (upper tracing) and the quickness of flow in the right coronary artery of the horse, simultaneously recorded (Rebatel's Fig. 3, page 25).

into the coronary artery, or to a sudden diminution of the peripheral resistance in the intramural vessels (p. 27)." To determine the origin of the second current, the tension and the quickness of flow in the coronary artery were recorded simultaneously. It was then seen that the tension curve was like that of every other artery, and presented no secondary rise or other feature that could account for the secondary augmentation in the quickness of flow. Thus led to a variation in the peripheral resistance, Rebatel concluded that the primary blood-wave

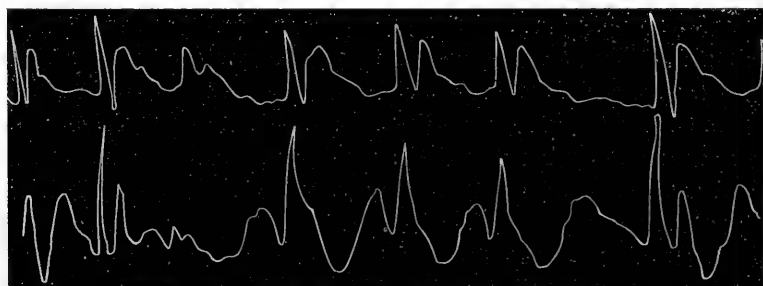


FIG. 2. Curves of the tension and quickness of flow in the right coronary artery of the horse, simultaneously recorded (Rebatel's Fig. 8, page 29).

penetrates with difficulty into the intramural branches during systole, because of their compression by the contracting cardiac muscle, but when the relaxation of the ventricle opens the peripheral vessels, the pent stream rushes suddenly forwards, and thus produces the second, or diastolic, rise in the curve of the hæmodromograph.

Rebatel himself does not accept this hypothesis unreservedly. His attitude is that of M. Marey,<sup>1</sup> who, on being shown the curves, admitted that the first proposition, namely, the filling of the coronary arteries in systole, is incontestable, while the second, namely, that the increase in quickness of flow is due to the opening of the intramural vessels by the relaxation of the ventricle is only "very probable and legitimately deduced." An analysis of Rebatel's tracings (Fig. 2) suggests that even this qualified approval was incautious. The extraordinary artificial irregularities in these curves at once attract attention. The curves are defaced by huge after-vibrations and inertia errors. The shock of the primary wave drives the writing levers far beyond the real maximum of the upstrokes; there is then a sharp rebound, which, in several instances, carries the writing points far below the correct level of the curve. Evidently the lever of the hæmodromograph, once set in motion by the sudden and violent changes in pressure and velocity consequent on the beat of the heart, has continued to swing. Serious as these faults are, they are by no means the chief reason for doubting the correctness of Rebatel's reasoning. According to him, the second augmentation is caused by the relaxation of the ventricle opening the compressed intramural vessels. But this relaxation occurs in the first half of the cardiac cycle, as shown by the position of the dicrotic notch in those of his tension curves that are written clearly enough to make the dicrotic notch visible. Hence, the maximum of the second augmentation, according to the hypothesis, should occur at the moment when the compressed vessels are opened by the swiftly relaxing ventricle, and not shortly before the ventricle contracts again, as in the curves before us. The delay cannot be explained by the sluggishness of the recording apparatus, for Rebatel assures us that the primary waves of tension and quickness are synchronous.

The form of the wave supposed to indicate a second augmentation of the rate of flow is still less reconcilable with Rebatel's hypothesis. The alleged increase in velocity quickly reaches its maximum, and is always succeeded by a rapid fall, greater in many of the tracings than the rise which precedes it. In the absence of any change in the blood-pressure either in the aorta or in the coronary arteries, it is impossible to understand how this slowing in the blood stream can take

<sup>1</sup> M. Marey, que nous remercions d'avoir bien voulu examiner nos tracés, admet, ainsi que nous, la première proposition comme incontestable, et la seconde comme très-probable et légitimement déduite (p. 31).

place. If a reservoir containing water kept at a constant level and provided with an elastic outflow tube is raised 130 cm. above the mouth of the tube, thus giving a constant pressure like that of the blood in the coronary arteries, shown to be constant by Rebatel's tension curves, and the mouth of the tube compressed, as the tubes of the coronary system are said by Rebatel to be compressed, and then released, while the water flowing out during two brief successive periods is measured, — it will be found that the outflow per unit of time, or in other words the velocity, is even a little greater in the second period than in the first. His second augmentation should, therefore, not have been followed by a marked slowing in the rate of flow.

Thus, seeing that Rebatel's second augmentation of velocity is not synchronous in his own curves with the relaxation said to be its cause, and perceiving that the form of the curve offered by him in evidence is physically improbable under the conditions premised by him, we may conclude that his results do not prove his assumption that the intramural vessels of the heart are compressed in systole.

I have spoken thus fully of Rebatel's work both because of its intrinsic interest and because his are the only recorded experiments that bear directly upon the problem in hand. It is true that Martin and Sedgwick,<sup>1</sup> ten years after Rebatel, recorded simultaneously curves of the blood-pressure in the carotid artery and in a branch of the left coronary artery; but their tracings were taken with a mercury manometer, and show nothing more than the synchronism in the primary pulse-wave, finer details being obscured by the inertia of the mercury.

## I.

My own observations upon the characters of the coronary pulse began Sept. 16, 1895, with the record of the pressure-curve in the carotid and left coronary arteries in the dog. It seemed *a priori* probable that variations in the peripheral resistance in the coronary arteries would be visible in the pressure-curve, provided it were written with a sensitive manometer.

The heart of a dog anæsthetized with ether was exposed, the ramus descendens of the left coronary artery ligated about two centimetres from its origin, and a cannula tied into the central end. The

<sup>1</sup> MARTIN, H. N., and W. T. SEDGWICK: *Journal of physiology*, 1882, iii. p. 165.

cannula was then connected by thick-walled but flexible rubber tubing to a glass tube, which led to a sensitive Hürthle membrane manometer, placed on the level of the artery. Evidently a manometer thus situated must receive the pressure-changes in the ramus circumflexus of the left coronary artery and in the branches given off by the ramus descendens in the first part of its course, *i. e.*, between its origin and the cannula. A second manometer recorded simultaneously the changes of pressure in the carotid artery. But the hope of securing a curve from the coronary arteries differing from the pressure-curve

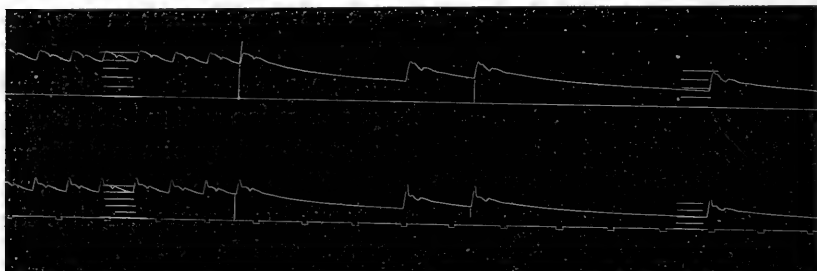


FIG. 3. Sept. 16, 1895. Curves of the blood-pressure in the left coronary artery (upper tracing) and the carotid artery (lower tracing) of the dog, recorded simultaneously. One half the original size. The horizontal line below each curve is the line of atmospheric pressure. In the case of the carotid artery, the atmospheric pressure line served also for the record of the time, in fifths of a second. The intervals of the graduation-scales correspond to a pressure of 20 mm. Hg. On raising the pressure in the Hürthle manometers to 100 mm. Hg, as here recorded, and then opening the chamber of the manometer to the pressure of the atmosphere, the writing points returned accurately to the line of atmospheric pressure, — this line in the pressure-scale being thus twice drawn. The vertical lines are synchronous ordinates. During the latter part of the curves, the heart was slowed by vagus excitation.

of other arteries was not realized. The most careful scrutiny of the two curves taken during the ordinary contractions and during the slowing of the heart by vagus excitation (see Fig. 3) failed to reveal any noteworthy difference, except that the pulse-wave reaches the coronary artery sooner than the carotid, depending of course on the nearness of the former vessel to the heart.

The first fully satisfactory evidence of the effect of the contraction of the ventricle on the flow of blood through the walls of the heart was secured during the writer's experiments on extirpated portions of the ventricle of the dog and cat. When a piece of the mammalian ventricle is kept beating by supplying it with defibrinated blood



through its nutrient artery at a constant pressure, each beat can be seen to force the blood out of the severed vessels in the margins of the fragment. The details of several of these experiments are as follows: —

*Experiment March 29, 1897.* A dog weighing 11 kilos, anaesthetized with morphia and ether, was bled from the left carotid artery, and the blood whipped, filtered through glass wool, and diluted with an equal volume of 0.8 per cent normal saline solution. Normal saline of the same strength was meanwhile allowed to flow into the right jugular vein. After a short interval, the dog was again bled from the carotid artery. A second injection of saline solution was followed by a third bleeding. The product of these bleedings was mixed, and placed in a reservoir at the temperature of the body. The heart was now extirpated, and a cannula tied into the ramus descendens of the left coronary artery not far from the apex of the left ventricle. That part of the apex which could be fed through the cannula was then excised. Both apex and basal portion fibrillated. The septum was removed. The piece of ventricle secured was 28 mm. in length (*i. e.* the direction from the base to the apex), 23 mm. broad opposite the end of the cannula, and 27 mm. broad at the somewhat flattened tip of the apex. The ventricle measured from base to apex 70 mm. The cannula was now connected with the blood reservoir and the apex perfused with blood. In a few moments regular and strong contractions set in. Curves were recorded with an ordinary muscle lever. The flow of blood from the veins was increased during each systole. The experiment was stopped after the apex had contracted one hour and forty minutes. During a part of this time the preparation was in a bath of blood at the temperature of the body.

*March 30, 1897.* On the morning of this day, a cannula was placed in the ramus descendens of a dog prepared as in the foregoing experiment, and most of the left ventricle and all of the right ventricle and septum except a fringe near the arteria descendens cut away. The portion remaining was fed through the cannula with defibrinated dog's blood, and beat strongly and at first quite regularly. It was observed that the outflow from the veins was increased at each systole. Distinct pulsations synchronous with the contractions of the heart-fragment were observed in the vena descendens at the point where it crosses the auriculo-ventricular groove. A cannula was tied into this vein, and a pulsation of the liquid in the cannula noted.

*April 5, 1897.* A pulse synchronous with systole was observed in the liquid in a cannula placed in the coronary artery of a piece of dog's ventricle fed with defibrinated blood from a reservoir at a constant pressure.

*April 9, 1897.* The circumflex area of the left ventricle of a cat's heart was fed with defibrinated cat's blood at a constant pressure through a cannula

placed in the ramus circumflexus. A vein on the surface of the ventricle was incised, and a little stream of normal saline solution allowed to flow over the opening, so as to prevent the blood collecting there. By this means a clear view of the wound in the vein and the escaping blood was secured. The discharge from the vein was then seen to be distinctly greater with each contraction of the ventricle. The superficial veins in a fragment of the auricle left attached to the preparation were observed to be nearly obliterated by each systole of the auricle. The pulse in these auricular veins could not have been caused by the rhythmic contractions of the coronary sinus, for the pulse in the veins continued after their separation from the sinus. Moreover, a similar pulse, noted in the superficial ventricular veins, ceased when the ventricle stopped beating, although the coronary sinus continued to contract.

The effect of the contraction of the heart on the contents of the intramural vessels can also be demonstrated in the living animal, as the next experiment will show.

*April 12, 1897.* A dog weighing 24 kilos was anaesthetized with morphia and ether, and the heart exposed by the resection of a part of the first five ribs on the left side. A branch of the vena descendens was incised about midway between the base and the apex of the ventricle, and a small stream of warm 0.8 per cent normal saline solution allowed to flow over the spot in order that the wound and the quantity of blood escaping from it might be readily seen. The vagus was now divided in the neck, and the peripheral end stimulated with induction shocks of such a strength that the ventricle was not continuously inhibited, but still gave occasional beats. Each time the ventricle contracted, the blood gushed from the vein. The increased outflow appeared absolutely synchronous with the contraction.

An eye-witness of this experiment could hardly have been persuaded that the gush of blood from the vein in systole was due to the transmission of the arterial pulse wave through the capillaries into the veins, yet it seemed advisable to answer this possible objection by direct experiment.

*July 22, 1897.* The experiment of April 12 was this day repeated, and again each contraction of the ventricle caused a greatly increased outflow from the vein. The vagus inhibition being prolonged, the heart swelled greatly, and the occasional contractions which broke through the inhibition were very strong. Each of these powerful contractions caused the blood to spurt from the vein. The heart was now excised, and the aorta connected with a reservoir of defibrinated dog's blood much diluted with 0.8 per cent

NaCl solution. The pressure in the reservoir was about 100 mm. Hg, so that, as soon as the connection with the aorta was made, the blood from the reservoir filled the artery, closed the semilunar valves, and passed through the coronary vessels. The perfused heart beat for a few minutes with considerable strength. With each beat the wound in the vein spurted, as an artery spurts when severed in the living animal.

The following experiments show that the squeezing of the vessels by the contracting muscle fibres makes itself evident in the arteries as well as in the veins. In this connection, it should be remarked once more, that the coronary arteries are "terminal arteries." In the absence of a collateral circulation, any pulsation observed in the distal segment of a coronary artery after its ligation is probably due to the compression of the intramural vessels by the contraction of the heart, and not to the transmission of an arterial pulse through collateral branches from other arteries.

*November 18, 1897.* A dog was anæsthetized with morphia and ether, and the heart exposed by the resection of the first five ribs on the left side. The ramus descendens was then ligated about 20 mm. from its origin. The artery was now incised 10–12 mm. distal to the ligature. A little blood escaped from the wound. On stimulating the vagus so that the ventricle contracted only occasionally, and allowing a small stream of warm normal saline solution to flow over the opening, it was possible to see plainly that each beat forced blood out of the artery. There was no visible delay between the beat and the outflow. The artery was now tied a few millimetres distal to the wound. The slight flow from the artery then ceased altogether, but during each systole a little blood appeared at the mouth of the wound in the artery.

The next day, a very high constant pressure was suddenly made in the aorta of a living dog, so that the semilunar valves were kept closed for a time, the pressure on their aortic side being greater than the maximum pressure in the left ventricle. The coronary circulation was fed during this time not by the beat of the ventricle but by the blood in the pressure-reservoir. Nevertheless, each beat of the ventricle forced blood out of the incision made in a coronary vein on the surface of the ventricle and out of a wound made in the arteria descendens distal to a ligature which had been placed around it. The details are as follows: —

*November 19, 1897.* The great vessels and heart of a dog anæsthetized with morphia and ether were exposed by resecting five ribs on the left and

three ribs on the right side and removing the upper part of the sternum. Cannulas were placed in the right and left carotid arteries. The right subclavian artery was ligated at its origin, and the left subclavian artery and the aorta prepared so that they could be clamped at the proper moment. The cannula in the left carotid artery was connected with a reservoir containing 0.8 per cent NaCl solution at a pressure of 140 mm. Hg. The cannula in the right carotid was connected to a mercury manometer, which showed a maximum pressure of 51 mm. Hg (the heart being rather feeble from long exposure). A vein on the surface of the left ventricle was now incised. The venous blood escaped from the wound in weak jets synchronous with the contractions of the ventricle, which were infrequent enough to permit the outflow to be seen distinctly. The stopcock between the carotid artery and the reservoir of saline solution under pressure was now opened and the left subclavian artery and the aorta clamped. The pressure in the manometer connected with the right carotid artery then rose to more than double its former maximum height. The semilunar valves were kept shut by this very high pressure in the aorta. The left ventricle, unable to open the semilunar valves, became greatly distended. An observation on the outflow from the incised artery and vein was made the moment the high pressure in the aorta closed the semilunar valves, before there could possibly have been time for the heart-beat to change sufficiently to overcome a pressure nearly three times as great as the former maximum arterial pressure, if indeed it could ever have done so. The blood still emerged from the vein in gentle systolic jets. The wound in the artery merely oozed, but the quantity escaping was distinctly greater in systole.

The emptying of the intramural vessels by the systolic squeeze of the fibres around them has been repeatedly observed in this Laboratory in the course of experiments on the extirpated heart of the cat, and has recently been admirably demonstrated by my friend, Mr. F. H. Pratt, by suspending a strip of the cat's heart, fed through one of the coronary arteries, in a large vessel of normal saline solution. The experiment is so instructive that it seems worth while to describe in this place a simple method by the aid of which the phenomena may be very easily shown.

A cat is anæsthetized with ether and cannulas placed in the left carotid artery and the right jugular vein. The animal is now bled from the artery. When the blood no longer flows except in drops, the artery is clamped, and 0.8 per cent NaCl solution at a temperature of 37° C. allowed to flow slowly into the jugular vein. When the blood vessels are well filled with saline solution, the cat is bled

again. The blood drawn in the first bleeding is diluted one half with the normal saline solution. The defibrinated blood mixture from both bleedings is then placed in a Mariotte tube, 30 cm. long and 3 cm. in diameter, of 190 c.c. capacity. The Mariotte tube opens below into a vertical glass tube about 5 mm. in diameter, on the end of which is a cannula provided with a stopcock. The cannula is inserted in the ramus descendens or the ramus circumflexus of the left coronary artery and all the heart cut away except that part of the ventricle supplied by the chosen artery. The fragment of the ventricle is now suspended in a very large beaker, filled with warm normal saline solution. When the Mariotte tube, the connecting tube, and the cannula are filled with the defibrinated blood, the height of the liquid column is about 65 cm., giving a blood-pressure of about 50 mm. Hg in the coronary artery. On opening the stopcock between the cannula and the upright tube, the blood circulates through the coronary artery and its branches, and the fragment of ventricle presently begins to beat. With each contraction the blood shoots from the severed vessels in the margins of the fragment some distance into the surrounding liquid, making a funnel-shaped cloud in the clear saline solution.

## II.

Having thus demonstrated the pressure which the muscular fibres in the heart exercise upon the intramural vessels during systole, it remains to consider to what extent this constriction and subsequent relaxation assist the flow of blood through the heart walls. That they do assist the flow of blood through the heart walls seems *a priori* probable; it is, indeed, difficult to imagine how the periodical squeezing of vessels communicating on the one hand with the aorta, a reservoir in which the pressure is always relatively very high, and on the other with outflow channels in which the pressure is always relatively very low, could fail to drive the blood towards the point of low pressure, *i. e.* into the veins. But these premises do not justify the conclusion that the systolic compression of the intramural vessels increases the total volume of the coronary circulation. This is quite another problem, and one which cannot be answered from the data thus far brought forward. It has just been demonstrated that the circulation through the intramural vessels is diminished during the contraction of the fibres around them. The emptying of the vessels

and their subsequent refilling is favored by this same rhythmic contraction. Which of these factors has the upper hand? Does the check which the circulation through the walls sustains during systole diminish the total volume of blood passing through the wall per minute, or is the lessening more than made up by the favorable factors — the emptying of the intramural vessels and their easier refilling? The experiments next to be described afford a partial answer to this question.

In February, 1896, while studying with Messrs. Magrath and Kennedy the relation of the volume of the coronary circulation to the frequency and force of the ventricular contraction in the isolated heart of the cat, I observed that the heart took more blood through the coronary arteries from a reservoir under constant pressure when contracting than when at rest. The same observation was made again, later in that year, when at work with Miss Hyde on the effect of the distention of the ventricle on the flow of blood through the walls of the heart. The fact is very well demonstrated by Fig. 4.

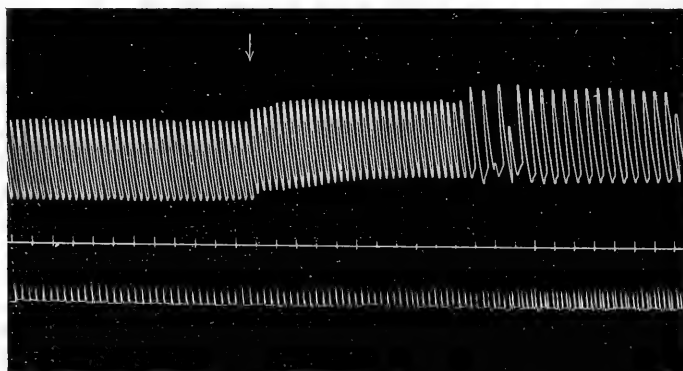


FIG. 4. Showing the increase in the volume of the coronary circulation consequent on an increase in the force of ventricular contraction. The uppermost tracing is the pressure in the left ventricle of the isolated heart of the cat, recorded by a Hürthle manometer; the next is the time, in seconds; and the lowermost is the record of the drops of blood flowing through the coronary vessels. The arrow points to the distention of the ventricle, which shortly calls forth beats of greater force. From an experiment performed with Miss I. H. Hyde.

In this experiment the extirpated heart of a cat was fed with warm defibrinated cat's blood from a reservoir at constant pressure through a cannula in the ascending aorta, all the branches of that vessel except the coronary arteries having previously been tied. The blood

passed from the coronary artery into the right ventricle, and thence through a glass tube, drop by drop, onto an aluminium plate fastened upon the lever of a Marey tambour.<sup>1</sup> The variation in the air pressure in the tambour occasioned by the falling drops was transmitted through a connecting tube to a second tambour, provided with a small chamber, thin membrane, and very light moving parts, and recorded by its writing lever upon the smoked paper of a kymograph. With this record of the number of drops of blood passing through the coronary vessels was written the pressure in the left ventricle, the cavity of which was filled with normal saline solution and connected with a sensitive Hürthle membrane manometer. A side branch led from this cannula to a Mariotte flask placed higher than the heart and filled with normal saline solution. When the stopcock leading to this flask was opened, the pressure in the left ventricle rose, as shown by the rise in the base line of the curve. After a few seconds the stimulus of the increased intracardiac pressure caused the ventricle to beat with greater force and the volume of the coronary circulation became greater, — and this in spite of a diminished frequency of contraction. Later, the pressure in the ventricle was lowered to that of the atmosphere, the ventricle contracted less vigorously, and the volume of the coronary circulation was correspondingly reduced.

A diminution in the volume of the coronary circulation in consequence of lessening the frequency of contraction is demonstrated by Fig. 5. The uppermost curve in this figure records the pressure in the left ventricle of the isolated heart of the cat, fed through the aorta and coronary vessels with defibrinated cat's blood at a constant pressure and temperature. The ventricle was filled with saline solution and connected with a Hürthle membrane manometer. The second curve was written by the armature of an electro-magnet placed in the primary circuit of a du Bois-Reymond inductorium. The heavy white line records the stimulation of the peripheral end of the vagus nerve with a weak induced current; the individual strokes of the armature are blended, owing to the slow speed of the smoked paper. The third curve marks the number of drops of blood flowing through the coronary vessels, the recording apparatus being that used for the experiment illustrated by Fig. 4. The fourth curve marks the time in seconds. The weak excitation of the vagus diminished the

<sup>1</sup> For the details of this method and a discussion of its sources of error, see MAGRATH and KENNEDY: *Journal of experimental medicine*, 1897, ii, p. 13.

frequency of ventricular contraction, but left the force unchanged. The volume of the coronary circulation lessened when the frequency of contraction lessened, and was restored with the restoration of the former frequency.

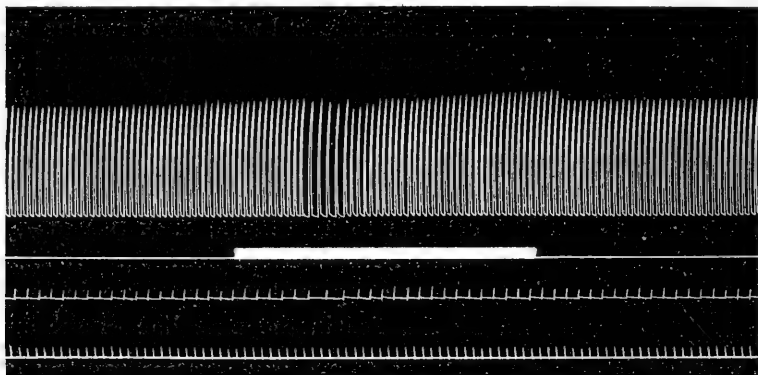


FIG. 5. March 26, 1896. Showing the lessening in the volume of the coronary circulation consequent on a lessening of the frequency of the heart-beat. The uppermost tracing is the pressure in the left ventricle of the isolated heart of the cat; the line below was drawn by the writing point of an electro-magnet placed in the primary circuit of the inductorium, the broad, white band indicating the duration of vagus stimulation; the next curve records the number of drops of blood passing through the coronary vessels; and the lowermost tracing is the time, in seconds. The weak excitation of the vagus finally lessens the frequency of contraction,—at once the volume of the coronary circulation is also lessened.

It would seem, then, that an increase in either the force or the frequency of the contractions of the heart increases the volume of blood passing through the coronary circulation by means of the periodical emptying of the intramural vessels,—yet it would not be prudent to accept this conclusion unreservedly. Two possible sources of error suggest themselves. The recorded changes in the volume of the coronary circulation may depend upon alterations in peripheral resistance in consequence of changes in the tonus of the heart muscle, or they may be due to changes in the vascular tonus in consequence of the action of vasomotor nerves. The first of these sources of error may be excluded with considerable certainty. The base line of the intraventricular pressure curves in Figs. 4 and 5 gives no evidence of changes in tonus; Fig. 5 is particularly convincing. The possible action of vasomotor nerves cannot be wholly excluded. Yet the pronounced synchronism between the changes



in frequency and the changes in the volume of the coronary circulation in Fig. 5 points toward a mechanical explanation, and seems to warrant the statement that the increase in the volume of the coronary circulation which accompanies an increase in the force or frequency of the heart-beat is probably to be explained by the periodical emptying of the intramural vessels by the contraction of the heart.

### III.

It is conceivable that the emptying of the intramural vessels by the contraction of the heart may favor the flow of blood through the heart walls in two ways: first, by the diminished resistance which the empty patulous vessels should offer to the inflow of blood from the aorta when the heart relaxes; and second, by the suction which might accompany the sudden expansion of the compressed vessels, — expanding either by virtue of their intrinsic elasticity, or because of the pull of the surrounding tissues upon their walls, as the heart quickly regains its diastolic form. It will be best to begin with the second problem, namely, the possible suction of the relaxing heart muscle.

The method by which this problem was attacked consists in suddenly connecting the distal portion of a coronary artery of the strongly beating heart with a small reservoir of blood at the atmospheric pressure. If each compression of the deeper branches of the artery were followed by an expansion sufficient to cause a noteworthy suction, the blood in the reservoir should be drawn into the artery; for this blood is the sole source of supply throughout the experiment, the "terminal" nature of the coronary arteries preventing any material backflow from collateral branches. It will be seen from the experiments about to be cited that no appreciable suction can be demonstrated in the larger coronary arteries, even when a very sensitive minimum valve is interposed between the artery and the reservoir in order to prevent the possible masking of the suction by rising pressures accompanying the contraction of the ventricle.

*April 14, 1897.* The heart and great vessels of a dog anesthetized with morphia and ether were exposed by the removal of a part of the chest wall. A glass cannula, 177 mm. long and 3.5 mm. in diameter, bent near the end as illustrated by Fig. 6, and furnished with a stopcock and a side branch leading to a minimum manometer, as shown in Fig. 7, was connected with a

reservoir containing warm defibrinated dog's blood, obtained in the manner described in the Exp. March 29, page 153. The pressure in the blood reservoir was maintained at a constant level of about 80 mm. Hg. (The exact reading of the mercury manometer connected with the reservoir was inadvertently omitted from the protocol.) The minimum valve and its manometer were filled with 0.8 per cent NaCl solution, and the cannula and the connecting tubes with defibrinated dog's blood. The long cannula was now rapidly passed through the innominate artery, aorta, and left coronary artery into the ramus circumflexus, which it filled completely, and the stopcock leading to the blood reservoir opened. The stopcock leading to the minimum manometer had previously been closed. The defibrinated blood entered the artery at about the normal temperature and pressure and maintained a satisfactory circulation. The heart continued to beat strongly and regularly. The blood reservoir was now suddenly shut off, and the stopcock leading to the minimum manometer as suddenly opened. The contents of the manometer passed slowly into the artery,

FIG. 6. Lower end of glass cannula for perfusing the ramus circumflexus in the living animal.

but on comparing the level of the liquid in the manometer, with that of the heart it was found that the manometer was higher than the heart. The slow emptying of the manometer may therefore have been due to gravity. The experiment shows at least that there is no strong suction, otherwise the manometer would have been emptied rapidly.

*April 15, 1897.* The foregoing experiment was repeated, but no suction could be demonstrated.

*April 16, 1897.* The experiment was varied by tying a cannula into the ramus descendens, opened for the purpose on the surface of the ventricle near the origin of the artery, and connecting this cannula with a minimum manometer, filled, as before, with normal saline solution. But no suction could be found.

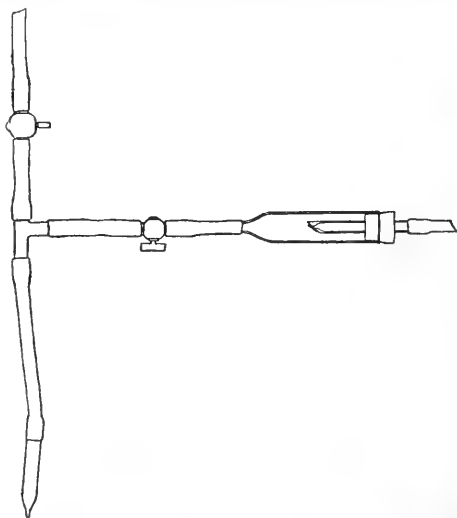


FIG. 7. Minimum valve and cannula, one-fourth actual size.

*April 23, 1897.* A cannula tied into the ramus descendens of a dog's heart was furnished with a T-tube, one limb of which led through a minimum manometer to a nearly horizontal glass tube filled with normal saline solution, while the other led to a reservoir from which the descendens area was supplied with warmed, defibrinated dog's blood at about the normal pressure (Fig. 7). While the heart was beating well, the descendens area being fed from the pressure flask, the latter was suddenly cut off and the stopcock leading to the minimum valve tube opened. There was no suction, although the conditions of the experiment were all favorable to its discovery.

Experiments similar to that of April 16 on the dog have been tried on four cat's hearts (Nov. 11-17), but also without finding any suction.

It should be remarked that these are all negative results. Against a single positive result they would be worthless. Yet I am obliged at present to conclude that the relaxation of the heart wall does not produce a suction in the larger coronary vessels.

Having failed to demonstrate any suction in the coronary arteries during the diastole of the heart, it is necessary to accept the alternative explanation of the favorable influence of the heart-beat on the flow of blood through the heart-walls, namely, the diminished resistance which the empty patulous vessels offer to the inflow of blood when the heart relaxes.

#### SUMMARY.

1. Curves of the blood-pressure in the carotid and the coronary artery, recorded simultaneously by two sensitive membrane manometers, reveal no noteworthy difference in the form of the pulse-wave.

2. The intramural branches of the coronary vessels are compressed by the contraction of the muscle fibres around them.

3. The volume of blood passing through the coronary vessels is increased by an increase in either the force or the frequency of the heart-beat.

4. It is probable that this increase in the volume of blood passing through the coronary vessels is accomplished largely through the periodical emptying of the intramural vessels by the systolic squeeze of the fibres around them.

5. The emptying of the intramural vessels by the contraction of the heart favors the flow of blood through the heart-walls chiefly by the diminished resistance which the empty patulous vessels offer to the inflow from the aorta when the heart relaxes.

6. The relaxation of the heart-walls does not produce a noteworthy suction in the larger coronary vessels.

# A FURTHER STUDY OF THE INFLUENCE OF ALCOHOL AND ALCOHOLIC DRINKS UPON DIGESTION, WITH SPECIAL REFERENCE TO SECRETION.<sup>1</sup>

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IN a previous paper<sup>2</sup> on the "Influence of Alcohol and Alcoholic Drinks upon the Chemical Processes of Digestion" it was carefully pointed out that any complete and satisfactory answer to the question "How do alcoholic fluids affect digestion?" cannot be obtained by any single line of experimentation, since the rate and extent of digestion may be modified in a variety of ways and through a variety of channels. Thus, due consideration must be given not only to the direct influence of alcoholic fluids upon the solvent or digestive power of the several digestive juices, but heed must also be given to the quantitative and qualitative modifications which the secretions themselves may undergo, as well as to variations in the rate of absorption and to the possible interaction of these and other factors. In our earlier paper, the data presented threw light only upon the character and extent of the influence exerted by various alcoholic fluids upon the purely chemical processes of digestion, *i. e.*, upon amyolysis and proteolysis. In the continuation of these studies during the past year our efforts have been directed mainly to acquiring a fuller knowledge of the action of alcoholic beverages upon secretion; and in so doing new data have been obtained which, it is hoped, will prove of value in explaining more fully the action of these fluids upon the whole process of digestion.

## SALIVARY SECRETION.

The current statements regarding the influence of alcohol on the secretion of saliva are confined to a brief reference to the direct

<sup>1</sup> Being a statement of some research work done for the Committee of Fifty for the Investigation of the Liquor Problem, and to be regarded as a preliminary report, contributing facts upon which a general discussion may in the future be undertaken by the Committee as a whole.

<sup>2</sup> CHITTENDEN and MENDEL: American journal of the medical sciences. 1896, January-April.

action on the flow into the mouth. Thus it is stated that almost coincident with the burning sensation caused by alcohol taken into the mouth, a copious flow of saliva begins, due to reflex stimulation of the glands through the nervous system.<sup>1</sup> We have performed experiments with the object of ascertaining (1) the possible variations in the amount of salivary flow due to the presence of alcoholic fluids in the mouth, psychical influences being eliminated so far as possible; (2) the character of the saliva thus secreted; (3) the influence upon secretion of alcoholic beverages introduced into the stomach. It seemed particularly desirable to investigate this latter phase in view of the asserted influence of irritating substances (vinegar, alcoholic extract of pepper, etc.) when introduced directly into the alimentary tract through a fistula. There is said to result under such conditions a reflex flow of saliva, the nervous impulses being transmitted through the vagus.<sup>2</sup>

**The Influence of Alcoholic Fluids introduced into the Mouth.**—In the following experiments the attempt was made to ascertain something as to the character and extent of the direct stimulation of the salivary glands provoked by the presence of alcoholic fluids in the mouth, as well as to determine what quantitative changes, if any, may be called forth in the composition of the secretion in this way. These experiments were made on both man and dogs. The method, in the first instance, consisted in taking into the mouth 10 c.c. of the fluid studied, and allowing it to remain there for an instant previous to swallowing it. The normal conditions were thus closely imitated, and reflex influences from the stomach not excluded. The head was now turned to one side and rested upon the arm, the saliva being allowed to collect in the cavity of the mouth. As the fluid accumulated it was from time to time, during fifteen to twenty minutes, allowed to flow out of a corner of the mouth into a measuring vessel. Movements of the jaws and tongue were carefully avoided and psychic stimulation was excluded as far as possible. The method, already recommended by Hofbauer,<sup>3</sup> was found to be reasonably satisfactory, and control trials showed that the quantities of saliva obtained within periods of fifteen or twenty minutes could be appropriately compared.

<sup>1</sup> Compare, for example, KÜHNE: *Lehrbuch der physiol. Chemie*, 1868, p. 2; LAUDER BRUNTON: *Disorders of digestion*, 1886, p. 143.

<sup>2</sup> OEHL: *Comptes rendus*, lix, p. 336, quoted by HEIDENHAIN, Hermann's *Handbuch der Physiologie*, 1883, v, p. 83.

<sup>3</sup> HOFBAUER: *Archiv für die ges. Physiol.*, 1897, lxxv, p. 503.

Of the saliva thus collected, 3-4 c.c. were taken for analysis. A weighed quantity was dried in a tared crucible on a water-bath and then for four or five hours at  $105^{\circ}\text{C.}$ , this time being found sufficient to bring crucible and contents to a constant weight. Total solids were thus determined. The crucible was then ignited, care being taken to prevent loss by volatilization of salts. The ash thus obtained is given as salts in the protocols, while the organic constituents were obtained by subtracting the amount of salts from the total solids. In some cases the amount of Cl in the ash was determined by the usual method of titration with weak silver nitrate solution. The analytical results are all expressed in percentages. The following figures serve to illustrate the results of a typical duplicate analysis: —

## SUBMAXILLARY SALIVA OF DOG.

	Water.	Total solids.	Organic constituents.	Salts.	Chlorine.
A.	98.99	1.01	0.80	0.21	0.042
B.	98.99	1.01	0.78	0.23	0.040

It is an observation easily verified, that the presence of a small quantity of strong alcohol or alcoholic beverage in the mouth excites a sudden flow of saliva. This acceleration in flow is, at most, a very brief one, and the rate of flow quickly returns to that pertaining to normal conditions, *i. e.*, absence of stimuli in the mouth. The stimulation in this case is not due merely to the mechanical action of the fluid introduced, nor is it a form of stimulation specific for alcohol alone, as our experiments on dogs have demonstrated. Thus, animals were anæsthetized with ether and chloroform through a tracheal cannula (thereby avoiding direct stimulation of salivary flow), a small dose of morphine, or a larger one of chloral, having been previously administered. A cannula was then introduced into one or both ducts of the submaxillary glands. A small wad of absorbent cotton moistened with the fluid to be studied was introduced with a forceps into the back of the mouth upon the tongue, and the flow of saliva from the ends of the cannulas noted. It was found by this method that water or weak sodium chloride solution (0.7 per cent) produced no further effect than the secretion of a drop or two of

SALIVARY EXPERIMENTS ON MAN.

	I.		II.		III.		IV.		V.		VI.		VI.		VIII.	
	water <i>a</i>	water <i>b</i>	water <i>a</i>	water <i>b</i>	water <i>a</i>	water <i>b</i>	water <i>a</i>	water <i>b</i>	water <i>a</i>	water <i>b</i>	water <i>a</i>	water <i>b</i>	water <i>a</i>	water <i>b</i>	water <i>a</i>	sherry <i>b</i>
Amount collected in c.c. per 10 minutes.	4.0	4.0	4.4	3.7	2.7	5.3	3.8	4.4	4.7	8.0	4.4	7.1	4.0	4.6	3.5	4.4
Water, per cent.	99.49	99.57	99.52	99.54	99.51	99.49	99.50	99.40	99.57	99.19	99.56	99.45	99.57	99.51	99.41	99.39
Total solids, per cent.	0.51	0.43	0.48	0.46	0.49	0.51	0.50	0.60	0.43	0.81	0.44	0.55	0.43	0.49	0.59	0.61
Organic constit- uents, per cent.	0.36	0.31	0.35	0.33	0.33	0.35	0.35	0.45	0.31	0.58	0.30	0.38	0.31	0.35	0.41	0.43
Salts, per cent.	0.15	0.12	0.13	0.13	0.16	0.16	0.15	0.15	0.12	0.23	0.14	0.17	0.12	0.14	0.18	0.18
Salts calculated on total solids, per cent.	29.0	29.0	28.0	28.0	32.0	32.0	30.0	25.0	29.0	28.0	31.0	31.0	28.0	28.0	30.0	29.0

saliva due to the mere mechanical action of introducing the wad; with increasing strengths of salt the secretion was decidedly accelerated, flowing readily after application of 20 per cent salt solution, the acceleration, however, being very brief in duration (5 min.). The buccal cavity could be swabbed out with water occasionally, the effect being a minimal one. It was found that *weak* alcohol, introduced in this way, provoked little, if any, flow; while stronger alcohol (50 per cent) gave rise to a transitory secretion, the stimulation in this case, however, being far more marked than can be produced by the indirect action of alcohol through the stomach. Thus, in one animal, in which the activity of the glands was found pronounced when a drop of dilute acetic acid was applied to the tongue, injection of 100 c.c. 50 per cent alcohol directly into the stomach, failed to provoke any reflex salivary flow in half an hour.

Turning now to the influence of alcoholic fluids upon the rate of flow and composition of the saliva in man, the accompanying experiments, by the method above indicated, may be cited (p. 167). The first two (I. and II.) show the results obtained with successive portions of water; in the following ones, a control experiment with water in each instance preceded the trial with the alcoholic fluid.

The alcoholic content of the fluids employed was as follows: Brandy, 47 per cent by vol.; gin, 51 per cent; sherry, 21 per cent.

From these figures it is seen that the results obtained with two successive portions of water scarcely differ from each other, the tendency however being towards decreased flow accompanied by decrease in dissolved material in the saliva. Interpreted in physiological terms, these results indicate that the second stimulation with water is, if anything, weaker than its predecessor. In decided contrast appear the results obtained with the alcoholic liquors. Here may be observed an increased flow of saliva, not pronounced, but accompanied by an increase in both organic and inorganic constituents. The effect is precisely analogous, both in composition and rate of flow, to that brought about by an increase in intensity of stimulation, when the salivary glands are electrically excited through their nerves.<sup>1</sup>

The following diagram represents in graphic form the results given in the preceding table, *i. e.* (1) the relative rate of flow induced by water and by the alcoholic fluid; (2) the content of solid matter,

<sup>1</sup> Cf. HEIDENHAIN: Archiv für die ges. Physiol., 1878, xvii, p. 7, and Hermann's Handbuch der Physiologie, v, p. 52.



together with the relative proportion of ash or inorganic matter and of organic matter as indicated by the loss on ignition.

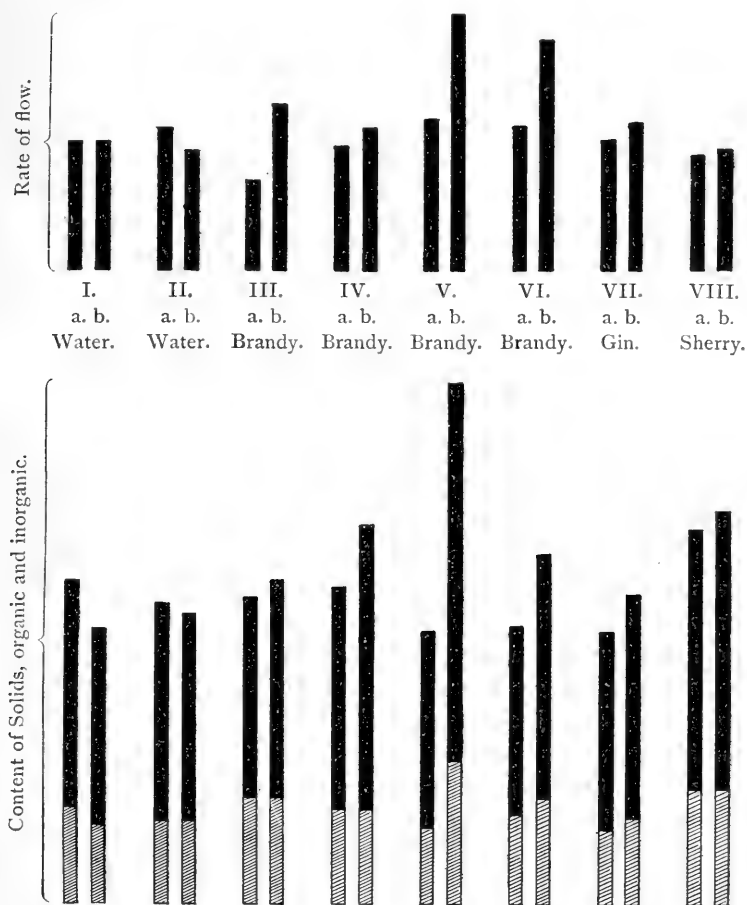


Diagram illustrating the relative influence of alcoholic fluids on the rate of secretion and composition of human saliva.

**The Influence of Alcoholic Liquors introduced directly into the Stomach.**

— In our experiments on the reflex stimulation of salivary flow, the attempt to produce a persisting secretion due to the presence of alcohol in the stomach was unsuccessful; nor have we been able to obtain evidence of an unusual flow of saliva under such circumstances in dogs with gastric fistulæ. It seemed desirable, however, to examine the possible direct influence of alcoholic fluids on the salivary glands

and the resulting secretion, when other factors were excluded as far as possible. In these experiments dogs of 10 to 18 kilos were used. Chloroform-ether mixture was employed to produce anæsthesia, and was administered through a tracheal tube in part of the experiments, the danger of respiratory difficulties resulting from salivary flow induced in the glands as a result of the ether stimulation being thus avoided. In the later stages of the experiments the alcohol introduced usually sufficed to maintain the animal in perfect quiet. Fredericq<sup>1</sup> has recommended the use of alcohol for producing narcosis in rabbits; it has been found quite satisfactory for this purpose in the dog, the effects passing off with relative rapidity.

A glass cannula, bent at the end, was tied in Wharton's duct (and occasionally a second cannula into the duct of the sublingual gland). The chordo-lingual nerve was ligatured and cut at some distance centrally to the point where the chorda tympani branches off to the glands. All secretion in the corresponding gland was thus stopped except during stimulation of the chorda, which was accomplished through raising the peripheral end of the cut nerve by the ligature and slipping hook electrodes under it. The interrupted current of a du Bois induction-coil with a single element was used as the stimulus. Saliva was collected in small graduated cylinders. Alcohol was introduced into the stomach by making an incision through the linea alba, etc., and injecting the fluid directly into the organ thus exposed by means of a large needle-pointed syringe. By careful avoidance of the larger gastric vessels, bleeding was minimal. The general course of the experiments was as follows: A distance between the primary and secondary coil of the inductorium was selected such as a preliminary trial showed to give a medium rate of flow. This stimulus was, so far as possible, kept constant throughout the experiment. The chorda was repeatedly stimulated for periods of one minute, followed by pauses of two minutes, during which the nerve was kept covered. In this way sufficient quantities of saliva for analysis were collected. Before collecting a sample of saliva under any given conditions, the six or seven drops first discharged were thrown away, and thus the fluid stored up from previous stimulation in the gland lumina, ducts, and cannula was avoided.<sup>2</sup> After collecting two or three control samples, the fluid to be considered (usually warmed slightly) was injected

<sup>1</sup> FREDERICQ: *Manipulations de physiologie*, p. 19.

<sup>2</sup> Cf. HEIDENHAIN: *Hermann's Handbuch der Physiologie*, v, p. 53; LANGLEY and FLETCHER: *Philosophical transactions*, 1889, clxxx, B., p. 112.

into the stomach in the manner already described, and this was followed by a pause of five minutes. The pulse was observed at frequent intervals to detect any possible influence on the heart's action and consequent blood-flow. The samples of saliva collected were analyzed in the manner already described. At the conclusion of the experiment, the animal was killed by bleeding, and the condition of the glands, as well as of the stomach and other organs, examined. The protocols of three typical experiments are given below.

**1, iii, 1897.** Dog. Weight 14 kilos. Chloroform and ether administered through tracheal tube during part of the experiment. Distance of secondary coil = 200 mm. Period of stimulation = 1 min., followed by a pause of 2 min.

	Time.	Amount saliva collected c.c.	Rate of secretion per min. c.c.	Water. per cent.	Total solids. per cent.	Organic matter. per cent.	Salts. per cent.	Chlorine. per cent.
I <sup>1</sup>	10.35	5.3	0.7	98.76	1.24	0.94	0.30	0.026
II	11.08	5.4	0.6	98.94	1.06	0.73	0.33	0.036
III	11.38	5.2	0.8	98.95	1.05	0.69	0.36	0.044
IV	11.56	4.0	0.8	98.90	1.10	....	....	0.048
	12.20	80 c.c. 50 per cent alcohol injected into stomach.						
V	12.35	4.8	0.8	98.96	1.04	0.69	0.35	0.047
VI	12.55	4.8	0.8	99.01	0.99	0.59	0.40	0.076
	1.15	100 c.c. 50 per cent alcohol injected into stomach.						
VII	1.21	4.9	0.8	99.05	0.95	0.59	0.36	0.055
VIII	1.42	6.0	1.0	99.05	0.95	0.60	0.35	0.060
IX	2.02	5.5	0.9	99.14	0.86	0.52	0.34	0.048
X	2.24	5.2	0.8	99.17	0.83	0.47	0.36	0.042
	2.53	100 c.c. 50 per cent alcohol injected into stomach.						
XI	2.58	4.5	0.6	99.07	0.93	0.63	0.30	0.034
XII	3.27	6.0	0.6	99.18	0.82	0.53	0.29	0.037
XIII	4.10	5.0	0.7	99.17	0.83	0.49	0.34	0.038

Dog killed. Stomach mucosa normal in appearance. Urinary bladder and gall bladder greatly distended. Stomach contents = 450 c.c., faintly acid in reaction, and containing 24.6 grams of alcohol. No food present.

<sup>1</sup> In this first period the distance of the secondary coil was 280 mm., but the stimulation was unsatisfactory.

22, iii, 1897. Bitch. Weight 10 kilos. Chloroform and ether administered during operation. Tracheotomy performed after operation. Distance of secondary coil = 240 mm. Period of stimulation = 1 min., followed by 2 min. pause.

	Time.	Amount saliva collected c.c.	Rate of secretion per min. c.c.	Water. per cent.	Total solids. per cent.	Organic matter. per cent.	Salts. per cent.	Chlorine. per cent.
I	11.30	4.6	1.1	98.68	1.32	1.03	0.29	0.032
II	11.42	4.7	0.9	98.70	1.30	0.96	0.34	0.074
III	11.57	4.0	0.7	98.84	1.16	0.73	0.43	0.146
	12.35	150 c.c. burgundy injected into stomach.						
IV	12.41	4.9	0.8	98.72	1.28	0.91	0.37	0.092
V	12.59	5.5	0.6	98.78	1.22	0.87	0.35	0.096
VI	1.29	4.7	0.7	98.91	1.09	0.82	0.27	0.071
	2.00	200 c.c. burgundy injected into stomach.						
VII	2.06	4.7	0.6	98.88	1.12	0.82	0.30	0.058
VIII	2.32	....	....	98.98	1.02	0.69	0.33	0.099

Dog killed; stomach contents = 190 c.c.; claret color; mucosa not inflamed. Contents contained 13.1 grams of alcohol. The burgundy used contained 5.2 per cent of alcohol.

Experiments of the character indicated by these protocols were carried out with alcohol in varying doses, whiskey, brandy, and wine, and control experiments with water were also made. In attempting to interpret the analytical data thus obtained in experiments extending over several hours it is necessary to bear in mind facts regarding salivary secretion which seem to be sufficiently established. Ludwig<sup>1</sup> showed that the submaxillary saliva secreted during stimulation of the chorda tympani undergoes a change in composition varying with the duration of the flow, the content of organic solids decreasing in far greater degree than the dissolved salts. Heidenhain<sup>2</sup> found that the percentage of salts in the saliva varies directly with the rate of secretion, quite independently of the state of the gland, the organic constituents, however, being influenced by the condition of the secreting organ as well as by the strength of stimulus and

<sup>1</sup> LUDWIG and BECHER: *Zeitschr. f. rat. Med.*, 1851, N. F. i, p. 278. Cf. also HEIDENHAIN: *Hermann's Handbuch der Physiologie*, v, pp. 47-49.

<sup>2</sup> HEIDENHAIN: *Archiv für die ges. Physiol.*, 1878, xvii, pp. 4 and 6.

12, iv, 1897. Bitch. Weight 9 kilos. Chloroform and ether during operation. Distance of secondary coil = 190 mm. Stimulation 1 min., followed by a pause of 2 min.

	Time.	Amount saliva collected c.c.	Rate of secretion per min. c.c.	Water. per cent.	Total solids. per cent.	Organic matter. per cent.	Salts. per cent.	Chlorine. per cent.
I	9.24	4.5	0.9	98.76	1.24	0.97	0.27	0.062
II	9.40	4.6	0.7	98.89	1.11	0.81	0.30	0.054
	10.40	100 c.c. distilled water injected into stomach.						
III	10.53	4.7	0.6	99.04	0.96	0.66	0.30	0.049
IV	11.21	5.0	0.5	99.09	0.91	0.60	0.31	0.060
	11.50	100 c.c. distilled water injected into stomach.						
V	11.56	4.5	0.5	99.30	0.70	0.54	0.16	0.024
VI	12.25	4.5	0.6	99.33	0.67	0.36	0.31	0.078
VII	12.51	4.6	0.7	99.39	0.61	0.36	0.25	0.063
	1.18	100 c.c. 50 per cent alcohol injected into stomach.						
VIII	1.23	5.7	0.7	99.35	0.65	0.36	0.29	0.067
IX	1.44	4.8	0.8	99.38	0.62	0.32	0.30	0.087
X	2.03	4.7	0.7	99.47	0.53	0.29	0.24	0.087
XI	2.25	4.7	0.6	99.47	0.53	0.22	0.31	0.097

Dog killed. Stomach mucosa normal. Contents = 100 c.c. No odor of alcohol.

resulting rate of secretion. These observations, verified by Werther<sup>1</sup> and by Langley and Fletcher,<sup>2</sup> have been extended by the latter investigators, who formulated the opinion that "the secretion of organic substances depends wholly, or almost wholly, upon the strength of the stimulus, whilst the secretion of water and of salts depends also upon the amount of blood flowing through the gland."<sup>3</sup> In view of the well-known fact that changes in the strength of the stimulus immediately bring about a change in both rate of secretion and composition of the saliva, we have attempted to maintain a constant stimulus throughout each series of observations by selecting some satisfactory distance of the secondary coil of the inductorium and by applying the electrodes as uniformly as possible. Owing to

<sup>1</sup> WERTHER: Archiv f. d. ges. Physiol., 1886, xxxviii, p. 293.

<sup>2</sup> LANGLEY and FLETCHER: *loc. cit.*, p. 152.

<sup>3</sup> *Ibid.*, p. 132.

the gradual decline in the irritability of the exposed nerve, the impossibility of applying the electrodes constantly in one position, and other unavoidable difficulties, ideal results cannot be obtained. However, the difficulties were present in every experiment and the results are therefore more or less comparable.

An examination of the data obtained in the manner above indicated shows no constant appreciable influence of alcohol or alcoholic fluids upon the *rate of secretion* of submaxillary (or sublingual) saliva under the influence of a constant external stimulus. Even large doses of alcohol, sufficient to produce prolonged narcosis, fail to check the salivary flow, a result in striking contrast to the effects which morphine may bring about when used in moderately large doses. We have not infrequently observed, in other experiments, an entire absence of salivary flow even with very strong stimuli, when morphine was unintentionally given in doses larger than were necessary to produce a mild narcosis. On the other hand, there is likewise an absence of any stimulating action on the glands, in our experiments; at least the slight variations in the rate of flow after alcohol is administered are no greater than those brought about by water alone (cf. third protocol above). On the *total solids* likewise, the presence of alcohol seems to exercise no noticeable influence. There is a tendency toward decrease in amount as the experiments progress; this decrease, however, is entirely confined to the *organic constituents* of the saliva, the *salts* remaining comparatively constant in amount, as can be seen in the protocols above. The decrease in organic substances is in no way to be attributed to alcohol, since it may be obtained with water alone (cf. protocol third), or in the course of any protracted salivary secretion. Nor is this decrease remarkable when it is remembered that a small gland weighing a few grams has furnished 50 to 75 grams of saliva in the course of three or four hours. The organic constituents of the cells must thus be exhausted somewhat more rapidly than the anabolic processes of the gland can replace them, while the salts are obtained with relative ease from the blood. Any effect upon the secretion of inorganic salts such as might result in accordance with Langley's law (cf. p. 173) was not observed. A large number of determinations of the alkalinity of the saliva (towards lacmoid) likewise failed to show any constant relations. It is interesting in this connection to note that the submaxillary saliva of the

dog was always found alkaline to phenolphthalëin, litmus, lacmoid, and methylorange. Mixed human saliva, like the bile of a number of animals, is almost always acid toward phenolphthalëin.<sup>1</sup>

#### GASTRIC SECRETION.

It has already been pointed out that in an accurate and complete study of the influence of alcohol and alcoholic drinks upon gastric digestion, no single line of experimentation can lead to full and concise results covering the whole ground of inquiry. It was therefore deemed advisable, for experimental purposes, to study the subject under several distinct heads, as (1) the influence of alcohol and alcoholic drinks upon the process of secretion; (2) upon the processes of absorption; (3) upon the motor functions of the alimentary canal; and (4) upon the purely chemical processes of gastric digestion. The last phase has already been considered at some length.<sup>2</sup>

The older announcements regarding the influence of alcohol are summarized in the statement that it is a strong stimulant of gastric secretion, and alcohol is recommended as a means of obtaining gastric juice from fistulæ in animals.<sup>3</sup> Larger doses are regarded as detrimental to the stomach, giving rise to transudation of alkaline fluid, — a process evidently pathological.<sup>4</sup> Gluzinski<sup>5</sup> found in experiments on man with brandy and dilute alcohol that these liquors gave rise, after a brief preliminary period, to the formation of a very active secretion rich in hydrochloric acid.

Likewise Wolff<sup>6</sup> states that cognac in small doses increases the secretion of hydrochloric acid, while in larger quantity it decreases the acidity of the gastric juice and retards peptone formation. The stomach fails to respond in a positive way, however, after the continued use of alcohol. While Klemperer<sup>7</sup> failed to note more than

<sup>1</sup> CHITTENDEN: The reactions of some animal fluids. *Science*, N. S., v, p. 902.

<sup>2</sup> CHITTENDEN and MENDEL: *loc. cit.*

<sup>3</sup> Cf. FRERICHS: Wagner's Handwörterbuch der Physiologie, 1846, iii, (1), p. 788; KÜHNE: Lehrbuch, pp. 28, 30; HEIDENHAIN: Hermann's Handbuch der Physiologie, v, p. 115.

<sup>4</sup> Cf. HEIDENHAIN: *loc. cit.*; LAUDER BRUNTON: Disorders of digestion, 1886, p. 144.

<sup>5</sup> GLUZINSKI: Deutsches Archiv f. klin. Med., 1886, xxxix, p. 405. See Jahresbericht für Thierchemie, 1886, xvi, p. 263.

<sup>6</sup> WOLFF: Zeitschr. f. klin. Med., 1889, xvi, p. 222; Jahresbericht f. Thierchemie, 1889, xix, p. 266.

<sup>7</sup> KLEMPERER: Zeitschr. f. klin. Med., 1890, xvii, Supp., p. 324; Centralbl. f. med. Wissen., 1891, p. 751.

a very slight increase in secretion resulting from moderate doses of alcohol, Blumenau<sup>1</sup> observed that 25–50 per cent alcohol introduced into the healthy human stomach acts as a secretory stimulant, bringing about an increased flow of gastric juice with rise of acidity after a period of 2–3 hours. More recently Brandl<sup>2</sup> has found in experiments on fistulous dogs that alcohol—as contrasted with water introduced with food stuffs into the stomach—brings about an unfailing, though not particularly large, increase in gastric secretion. With repeated and increasing doses of alcohol, Haan<sup>3</sup> has further observed an augmentation of acidity in the dog, followed by a diminution in the amount of secretion and a gradual decline in acidity after several doses.

In our first series of experiments on gastric secretion, attention was directed to the volume and acidity resulting from the introduction of alcoholic fluids into the stomach, independently of any stimulating action due to food simultaneously introduced. Dogs in fasting condition were employed in every instance, and morphine sulphate (introduced subcutaneously) followed by chloroform-ether was used preparatory to operative interference. The method consisted in ligating the duodenum just beyond the pylorus and then introducing a definite volume of the fluid to be examined into the empty stomach in the manner already indicated in previous experiments. In several cases, dogs with gastric fistulæ were employed. The abdomen was quickly sewed up after this operation, chloroform-ether stopped, and the animal allowed entire freedom of movement. The liquid employed was ordinarily warmed gently to avoid the asserted stimulating action of cold fluids on the gastric mucosa.<sup>4</sup> Ligations of the œsophagus and œsophageal fistulæ were avoided, since a somewhat extended experience with gastric fistula dogs, as well as the experiments about to be described, have convinced us, in agreement with Heidenhain's observations,<sup>5</sup> that under ordinary circumstances, *i. e.* in the absence of unusual stimuli (and with slightly narcotized animals) the amount of saliva secreted is small at most, and fails to induce any pronounced secretion in the stomach.<sup>6</sup> Further, we have

<sup>1</sup> BLUMENAU: *Therapeutische Monatshefte*, 1890, v, p. 504; *Jahresbericht f. Tierchemie*, 1891, xxi, p. 212.

<sup>2</sup> BRANDL: *Zeitschr. f. Biologie*, 1892, xxix, p. 304.

<sup>3</sup> HAAN: *Comptes rendus de la société de biologie*, 1895, ii, p. 817.

<sup>4</sup> Cf. KÜHNE: *Lehrbuch der physiol. Chemie*, p. 28.

<sup>5</sup> Hermann's *Handbuch*, v, p. 112.

<sup>6</sup> Compare also the experiment described on page 168.



found that an unusual flow of saliva is at once readily detected by the physical character of the stomach contents, *e. g.* frothing, etc. Furthermore, the conditions of our experiments were intended to approach those normally obtaining in the body as nearly as possible; and finally, a sufficient number of control experiments in which water was introduced into the stomach, have left no doubt as to the validity of the method. At the end of three to four hours—a period shown by our experiments to cover the digestion time of a test meal for the dog—the animal was bled to death, the œsophagus ligated at the lower end, the stomach removed from the body, wiped free from blood, and the contents discharged into a graduated vessel. In the fluid thus obtained total acidity, free and combined HCl, and acid reacting salts were determined by the method of Töpfer;<sup>1</sup> alcohol was estimated, when present, in the distillate from a definite portion of the gastric contents, by the pycnometer method; total solids were determined by drying a weighed quantity of fluid in a tared crucible at 100–105 C°. Protocols follow:—

**A. Control Experiments with Water:—**

- I.** 31 v, 1897. Dog, with gastric fistula, well healed. Weight 21 kilos. Fluid removed completely through fistula.

Introduced 200 c.c. *distilled water* at 10.50 A. M.

Contents removed at 1.55 P. M. =  $3\frac{1}{2}$  hrs.

Volume of fluid recovered from stomach = 160 c.c. = **80 per cent** of original volume.

Analysis of the contents gave:

Total acidity . . . . .	0.203 per cent. <sup>2</sup>
Free HCl . . . . .	0.192
Loosely combined HCl . . . .	0.002
Salts . . . . .	0.009
Total solids . . . . .	0.624

- II.** 28 vi, 1897. Dog, with gastric fistula, well healed. Weight 25 kilos. Fluid removed completely through fistula.

Introduced 135 c.c. *distilled water* at 11 A. M.

Contents removed at 1.45 P. M. =  $2\frac{3}{4}$  hrs.

Volume of fluid recovered from stomach = 110 c.c. = **81 per cent** of original volume.

Analysis of the contents gave:

Total acidity . . . . .	0.274 per cent.
Free HCl . . . . .	0.241
Loosely combined HCl . . . .	0.018
Salts . . . . .	0.015
Total solids . . . . .	0.77

<sup>1</sup> TÖPFER: Zeitschr. f. physiol. Chemie, 1894, xix, p. 104.

<sup>2</sup> Expressed as HCl in all the experiments.

## III. 24 v, 1897. Dog. Weight 7.7 kilos.

Introduced 125 c.c. *distilled water* at 10 A. M.Contents removed at 1.50 P. M. =  $3\frac{5}{8}$  hours.Volume of fluid recovered from stomach = 114 c.c. = **91 per cent** of original volume.

Analysis of the contents gave:

Total acidity . . . . .	0.094 per cent.
Free HCl . . . . .	0.065
Loosely combined HCl . . .	0.004
Salts . . . . .	0.025
Total solids . . . . .	0.47

## IV. 29 v, 1897. Dog. Weight 14.5 kilos.

Introduced 200 c.c. *distilled water* at 9.30 A. M.Contents removed at 1.15 P. M. =  $3\frac{3}{4}$  hrs.Volume of fluid recovered from stomach = 206 c.c. = **103 per cent** of original volume.<sup>1</sup>

Analysis of the contents gave:

Total acidity . . . . .	0.047 per cent.
Free HCl . . . . .	0.040
Loosely combined HCl . . .	0.004
Salts . . . . .	0.003
Total solids . . . . .	0.50

## V. 2 vi, 1897. Dog. Weight 10.5 kilos.

Introduced 125 c.c. *carbonated water* at 9 A. M.Contents removed at 12.45 P. M. =  $3\frac{3}{4}$  hrs.Volume of fluid recovered from stomach = 125 c.c. = **100 per cent** of original volume.

Analysis of the contents gave:

Total acidity . . . . .	0.191 per cent.
Free HCl . . . . .	0.152
Loosely combined HCl . . .	0.014
Salts . . . . .	0.025
Total solids . . . . .	0.55

In this experiment the  $\text{CO}_2$  was completely absorbed.

## VI. 1 vii, 1897. Dog. Weight 10 kilos.

Introduced 76 c.c. of 2 per cent *dextrose* solution at 9.10 A. M.Contents removed at 12.40 P. M. =  $3\frac{1}{2}$  hrs.Volume of fluid recovered from stomach = 68 c. c. = **90 per cent** of original volume.

Analysis of the contents gave:

Total acidity . . . . .	0.072 per cent.
Free HCl . . . . .	0.047
Loosely combined HCl . . .	0.007
Salts . . . . .	0.018

<sup>1</sup> A small quantity of saliva doubtless found its way into the stomach, as the dog salivated somewhat at the beginning of the operation and the stomach contents had a frothy appearance.

**B. Experiments with strong Ethyl Alcohol: —**

VII. 17 v, 1897. Dog. Weight 23 kilos.

Introduced 200 c.c. of 37 per cent *alcohol* at 10.45 A. M.

Contents removed at 2.15 P. M. =  $3\frac{1}{2}$  hrs.

Volume of fluid recovered from stomach = 407 c.c. = **203 per cent** of original volume.

Analysis of the contents gave:

Total acidity . . . . .	0.164 per cent.
Free HCl . . . . .	0.112
Loosely combined HCl . . .	0.043
Salts . . . . .	0.009

VIII. 31 v, 1897. Dog. Weight 21 kilos. Gastric fistula well healed.

*Contrast experiment with water and alcohol.*

a. The first part of this experiment has been described under I. p. 177.

β. After discharge of previous stomach contents completely through fistula, 200 c.c.  $37\frac{1}{2}$  per cent *alcohol* were introduced into the stomach through fistula at 1.55 P. M.  
Contents removed at 5 P. M. =  $3\frac{1}{2}$  hrs.

Volume of fluid recovered from stomach = 460 c.c. = **230 per cent** of original volume.<sup>1</sup>

Analysis of the contents gave:

Total acidity . . . . .	0.220 per cent.
Free HCl . . . . .	0.164
Loosely combined HCl . . .	0.011
Salts . . . . .	0.045
Total solids . . . . .	0.987

**C. Experiments with weak (5 per cent) Ethyl Alcohol: —**

IX. 24 vi, 1897. Bitch. Weight 8 kilos.

Introduced 100 c.c. 5 per cent *alcohol* at 10.45 A. M.

Contents removed at 2 P. M. =  $3\frac{1}{4}$  hrs.

Volume of fluid recovered from stomach = 110 c.c. = **110 per cent** of original volume.

Analysis of the stomach contents gave:

Total acidity . . . . .	0.119 per cent.
Free HCl . . . . .	0.086
Loosely combined HCl . . .	0.011
Salts . . . . .	0.022
Total solids . . . . .	0.69

X. 8 vi, 1897. Bitch. Weight 7.3 kilos.

Introduced 110 c.c. 4.8 per cent *alcohol* at 9 A. M.

Contents removed at 12.45 P. M. =  $3\frac{3}{4}$  hrs.

Volume of fluid recovered from stomach = 135 c.c. = **123 per cent** of original volume.

Analysis of the stomach contents gave:

Total acidity . . . . .	0.202 per cent.
Free HCl . . . . .	0.148
Loosely combined HCl . . .	0.021
Salts . . . . .	0.033

<sup>1</sup> A post-mortem examination showed that the stomach contents could be completely discharged through the fistula by the method adopted.

The results of the foregoing experiments, expressed in percentages, are combined in the following table.

A. With water.	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
I	80	0.203	0.002	0.192	0.009	0.62
II	81	0.274	0.018	0.241	0.015	0.77
III	91	0.094	0.004	0.065	0.025	0.47
IV	103	0.047	0.004	0.040	0.003	0.50
V	100	0.191	0.014	0.152	0.025	0.55
VI	90	0.072	0.007	0.047	0.018	....
Average.	90.8	0.147	0.008	0.123	0.016	0.58

B. With strong alcohol.	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
VII	203	0.164	0.043	0.112	0.009	....
VIII	230	0.220	0.011	0.164	0.045	0.99
Average.	216.5	0.192	0.027	0.138	0.026	0.99

C. With weak alcohol.	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
IX	110	0.119	0.011	0.086	0.022	0.69
X	123	0.202	0.021	0.148	0.033	....
Average.	116.5	0.160	0.016	0.117	0.027	0.69

A glance at the data presented leaves little doubt as to the pronounced stimulating action of pure ethyl alcohol upon gastric secre-

tion, even with solutions of only five per cent strength. The effect is not merely one characterized by the discharge of water into the stomach cavity, but gives evidence of a true secretory process. Thus, the volume of fluid found after introduction of water into the stomach is not increased, there being rather a tendency in the opposite direction. Edkins,<sup>1</sup> v. Mering,<sup>2</sup> and others have shown that the absorption of water from the stomach is practically *nil*, while the absorption of alcohol goes on quite rapidly. In our own experiments, the alcohol used had entirely disappeared from the stomach in the course of the experiments; the question of absorption will, however, be referred to in another connection. With five per cent alcohol the increase in the volume of the gastric contents is noticeable, becoming very pronounced with the stronger percentages of alcohol. The increase in total solids gives confirmation of stimulated secretion, as does also the increase in acidity. It must be remembered, further, that the increase in acidity shown by the figures is a relative one; expressed absolutely in grams, the total acid secreted is obviously increased in far greater degree than the percentage figures indicate. The specific action of alcohol is strikingly shown in Experiment VIII., in which the conditions permitted of comparative experiments with water and alcohol on the same animal, with the following results:—

COMPARISON OF THE TWO EXPERIMENTS (VIII.  $\alpha$ ,  $\beta$ ).

Fluid introduced in stomach.	Fluid recovered from stomach after 3 hours.	Relative volume. per cent.	Total acidity.	Free HCl.	Loosely combined HCl.	Salts.	Total solids.
200 c.c. water	160 c.c.	80	0.203	0.192	0.002	0.009	0.624
200 c.c. alcohol } (37½ per cent.) }	460 c.c.	230	0.220	0.164	0.011	0.045	0.987

A comparison of the proteolytic activity of the two secretions by Grützner's carmine-fibrin method showed a decidedly greater digestive power in the case of the "water" secretion. Much stress cannot be placed, however, on a single experiment. The gastric fluids obtained in the experiments with alcohol possessed strong proteolytic properties in every case examined.

<sup>1</sup> EDKINS: Journal of physiology, 1892, xiii, p. 445.

<sup>2</sup> v. MERING: Verhandlungen des XII. Congresses f. innere Medicin, Wiesbaden, 1893; Therapeutische Monatshefte, 1893, vii, p. 201.

In view of this pronounced action of alcohol on gastric secretion it seemed desirable to ascertain something more definite regarding the way in which this process is provoked. The control experiments with water gave evidence that the mere contact of the fluid with the stomach mucosa could not be the cause of gastric stimulation. It will be remembered that even vigorous mechanical stimulation or irritation ordinarily fails to yield more than a few grams of secretion,<sup>1</sup> — an observation in decided contrast to the phenomena of gastric flow during the presence of digestible materials in the stomach. The following experiments throw light on the question raised: —

- XI.** 25 v, 1897. Dog. Weight 23 kilos. The intestine was ligatured just beyond the pylorus. Another ligature was applied below the point of entrance of the duct of Wirsung. 20 c.c. of 60 per cent alcohol were injected into the lumen of the intestine between these ligatures, while 105 c.c. of 60 per cent alcohol were introduced into the intestine beyond the second ligature. Then

Introduced 200 c.c. *water* into stomach at 10.45 A. M.

Contents removed at 2.30 P. M. =  $3\frac{3}{4}$  hrs.

Volume of fluid recovered from stomach = 260 c.c. = **130 per cent** of original volume.

Analysis of stomach contents gave:

Total acidity . . . . .	0.241 per cent.
Free HCl . . . . .	0.213
Loosely combined HCl . . .	0.002
Salts . . . . .	0.026

- XII.** 28 v, 1897. Bitch. Weight 28 kilos. Intestine ligatured just beyond the pylorus. Another ligature was applied below the point of entrance of the duct of Wirsung. 125 c.c. of 60 per cent alcohol were injected into the lumen of the intestine below the second ligature,<sup>2</sup> then

Introduced 200 c.c. *water* into stomach at 11 A. M.

Contents removed at 2.45 P. M. =  $3\frac{3}{4}$  hours.

Volume of fluid recovered from stomach = 375 c.c. = **187.5 per cent** of original volume.

Analysis of stomach contents gave:

Total acidity . . . . .	0.333 per cent.
Free HCl . . . . .	0.306
Loosely combined HCl . . .	0.004
Salts . . . . .	0.023
Total solids . . . . .	0.30

<sup>1</sup> Cf. TIEDEMANN and GMELIN: Die Verdauung nach Versuchen, 1831, p. 92; SCHIFF: Leçons sur la physiologie de la digestion, ii, p. 244.

<sup>2</sup> The return of alcoholic fluid into the stomach was thus absolutely prevented.

SUMMARY OF RESULTS OF EXPERIMENTS.

No.	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
XI	130.0	0.241	0.002	0.213	0.026	....
XII	187.5	0.333	0.004	0.306	0.023	0.30
Average.	158.5	0.287	0.003	0.259	0.024	0.30

From these data it seems clear that a stimulation of the gastric glands may take place, independently of any *direct* gastric irritation, in consequence of the influence of alcohol absorbed from the intestine. The volume of the fluid in the stomach increased relatively far more than when five per cent alcohol was introduced directly into the stomach (cf. Experiments IX., X., p. 179). The composition of the fluid (high acidity, free HCl, total solids) likewise gives evidence of active secretion, while the fluid was found to be strongly proteolytic. The absorption of the alcohol was complete in these experiments; and when it is remembered how quickly alcohol is distributed and disappears in the body, the actual amount reaching the gastric glands must have been relatively small, or at least must have acted during a brief period only. It seems probable, therefore, that there occurs here an indirect stimulation quite comparable to that resulting after absorption of peptone from the alimentary tract, and it is interesting to note by way of comparison that Khigine,<sup>1</sup> in his experiments upon the isolated fundus of the dog, found that the acidity of the secretion after absorption of digestion products runs parallel to a certain degree with the increase in volume. Whether the absorbed alcohol acts directly upon elements of the gastric mucosa (Heidenhain's "secondary secretion"), or becomes a stimulus to specific secretory nerve fibres (Khigine), we are unable at present to decide.<sup>2</sup>

In connection with this "secondary" secretion of gastric juice due to the presence of alcohol in the small intestine, it is to be noted that Macfadyen, Nencki, and Sieber<sup>3</sup> found among the bacteria normally

<sup>1</sup> KHIGINE: Archives des sciences biologiques, St. Petersbourg, 1895, iii, p. 461.

<sup>2</sup> Cf. HOWELL: American text-book of physiology, 1896, p. 182.

<sup>3</sup> MACFADYEN, NENCKI, and SIEBER: Archiv f. experimentelle Pathologie und Pharmakologie, 1891, xxviii, p. 311.

present in this portion of the alimentary canal species which give rise to a production of ethyl alcohol from carbohydrates ingested.

D. **Experiments with Alcoholic Beverages.**— It might naturally be assumed that the action of the various alcoholic beverages on gastric secretion would be similar, qualitatively, to that of their common constituent ethyl alcohol. Previous investigation, however, has shown that the influence of these liquors on the purely chemical processes of digestion is not necessarily proportionate to their content of alcohol,<sup>1</sup> hence it seemed desirable to study the effect of a number of typical liquors on secretion, by the method of the previous experiments. This we have done with the following results:—

**XIII.** 21 vi, 1897. Dog. Weight 10.7 kilos.

Introduced 50 c.c. **sherry** + 25 c.c. *water* (14 per cent alcohol) at 10.20 A. M.

Contents removed at 2.15 P. M. =  $3\frac{1}{2}$  hrs.

Volume of fluid recovered from stomach = 160 c.c. = **213 per cent** original volume.

Analysis of stomach contents gave:

Total acidity . . . . .	0.367 per cent.
Free HCl . . . . .	0.300
Loosely combined HCl . . .	0.020
Salts . . . . .	0.047
Total solids . . . . .	1.72

**XIV.** 2 vi, 1897. Dog. Weight 18.5 kilos.

Introduced 50 c.c. **whiskey** + 100 c.c. *water* (16 per cent alcohol) at 11.15 A. M.

Contents removed at 3 P. M. =  $3\frac{3}{4}$  hours.

Volume of fluid recovered from stomach = 320 c.c. = **213 per cent** original volume.

Analysis of stomach contents gave:

Total acidity . . . . .	0.382 per cent.
Free HCl . . . . .	0.346
Loosely combined HCl . . .	0.011
Salts . . . . .	0.025
Total solids . . . . .	0.42

**XV.** 3 vi, 1897. Bitch. Weight 8 kilos.

Introduced 125 c.c. **Hochheimer** (13.3 per cent alcohol) at 10 A. M.

Contents removed at 1.45 P. M. =  $3\frac{3}{4}$  hrs.

Volume of fluid recovered from stomach = 140 c.c. = **112 per cent** original volume.

Analysis of stomach contents gave:

Total acidity . . . . .	0.230 per cent.
Free HCl . . . . .	0.165
Loosely combined HCl . . .	0.038
Salts . . . . .	0.027

<sup>1</sup> CHITTENDEN and MENDEL: *loc. cit.*



**XVI.** 28 vi, 1897. Dog. Weight 25 kilos. Gastric fistula well healed.

*Contrast experiment with water and wine.*

- a. The first part of this experiment has been described under II. p. 177.
- β. After complete discharge of previous stomach contents through the fistula, 135 c.c. **white wine** were introduced into stomach through fistula at 1.45 P. M.

Contents removed at 4.30 P. M. =  $2\frac{3}{4}$  hrs.

Volume of fluid recovered from stomach = 170 c.c. = **126 per cent** original volume.

Analysis of stomach contents gave :

Total acidity . . . . .	0.425 per cent.
Free HCl . . . . .	0.342
Loosely combined HCl . .	0.018
Salts . . . . .	0.065
Total solids . . . . .	1.79

**XVII.** 23 vi, 1897. Dog. Weight 12.3 kilos.

Introduced 125 c.c. **claret** (5.15 per cent alcohol) at 9.30 A. M.

Contents removed at 1.30 P. M. = 4 hrs.

Volume of fluid recovered from stomach = 225 c.c. = **180 per cent** original volume.

Analysis of stomach contents gave :

Total acidity . . . . .	0.373 per cent.
Free HCl . . . . .	0.324
Loosely combined HCl . .	0.025
Salts . . . . .	0.024
Total solids . . . . .	1.90

**XVIII.** 18 vi, 1897. Bitch. Weight 10.2 kilos.

Introduced 100 c.c. **lager beer** (4 to 5 per cent alcohol) at 10.20 A. M.

Contents removed at 2.15 P. M. =  $3\frac{1}{2}$  hours.

Volume of fluid recovered from stomach = 110 c.c. = **110 per cent** original volume.

Analysis of stomach contents gave :

Total acidity . . . . .	0.357 per cent.
Free HCl . . . . .	0.241
Loosely combined HCl . .	0.064
Salts . . . . .	0.052
Total solids . . . . .	9.26

**XIX.** 23 vi, 1897. Dog. Weight 10 kilos.

Introduced 100 c.c. **lager beer** (4.5 per cent alcohol) at 10.10 A. M.

Contents removed at 2 P. M. =  $3\frac{5}{8}$  hrs.

Volume of fluid recovered from stomach = 125 c.c. = **125 per cent** original volume.

Analysis of stomach contents gave :

Total acidity . . . . .	0.241 per cent.
Free HCl . . . . .	0.169
Loosely combined HCl . .	0.032
Salts . . . . .	0.040
Total solids . . . . .	5.51

**XX.** 14 vi, 1897. Dog. Weight 14 kilos.Introduced 150 c.c. **porter** (3.75 per cent. alcohol) at 9.45 A. M.Contents removed at 1.30 P. M. =  $3\frac{3}{4}$  hrs.Volume of fluid recovered from stomach = 195 c.c. = **127 per cent** original volume.

Analysis of stomach contents gave :

Total acidity . . . . .	0.371 per cent.
Free HCl . . . . .	0.320
Loosely combined HCl . . .	0.036
Salts . . . . .	0.015
Total solids . . . . .	2.19

**XXI.** 7 vi, 1897. Bitch. Weight 8.5 kilos.Introduced 125 c.c. **lager beer** (4.7 per cent alcohol) at 10.15 A. M.Contents removed at 2.10 P. M. =  $3\frac{1}{2}$  hrs.Volume of fluid recovered from stomach = 285 c.c. = **228 per cent** original volume.

Analysis of stomach contents gave :

Total acidity . . . . .	0.378 per cent.
Free HCl . . . . .	0.308
Loosely combined HCl . . .	0.016
Salts . . . . .	0.054
Total solids . . . . .	2.88

**XXII.** 14 vi, 1897. Dog. Weight 8.2 kilos.Introduced 150 c.c. **porter residue**<sup>1</sup> at 11.30 A. M.Contents removed at 3.15 P. M. =  $3\frac{1}{4}$  hrs.Volume of fluid recovered from stomach = 135 c.c. = **90 per cent** original volume.

Analysis of stomach contents gave :

Total acidity . . . . .	0.352 per cent.
Free HCl . . . . .	0.280
Loosely combined HCl . . .	0.014
Salts . . . . .	0.058
Total solids . . . . .	2.29

**XXIII.** 9 vi, 1897. Dog. Weight 10 kilos.Introduced 130 c.c. **lager beer residue**<sup>2</sup> at 10.30 A. M.

Contents removed at 2.30 P. M. = 4 hrs.

Volume of fluid recovered from stomach = 175 c.c. = **134 per cent** original volume.

Analysis of stomach contents gave :

Total acidity . . . . .	0.346 per cent.
Free HCl . . . . .	0.270
Loosely combined HCl . . .	0.038
Salts . . . . .	0.038
Total solids . . . . .	6.80

<sup>1</sup> The residue left on evaporation of 150 c.c. porter, dissolved in 150 c.c. distilled water.<sup>2</sup> Residue from evaporation of 130 c.c. beer, dissolved in 130 c.c. water.

For the sake of comparison these data are contrasted in the following table: —

	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
XIV. Whiskey + H <sub>2</sub> O (16% alcohol)	213	0.382	0.011	0.346	0.025	0.42
XIII. Sherry + H <sub>2</sub> O (13% alcohol)	213	0.367	0.020	0.300	0.047	1.72
XV. White wine . . . (13% alcohol)	112	0.230	0.038	0.165	0.027	....
XVI. White wine . . . (13% alcohol)	126	0.425	0.018	0.342	0.065	1.79
XVII. Claret . . . . . (10% alcohol)	180	0.373	0.025	0.324	0.024	1.90
XVIII. Beer . . . . . (+7% alcohol)	110	0.357	0.064	0.241	0.052	9.26
XIX. Beer . . . . . (+% alcohol)	125	0.241	0.032	0.169	0.040	5.51
XXI. Beer . . . . . (+7% alcohol)	228	0.378	0.016	0.308	0.054	2.88
XXIII. Residue of Beer (like XXI.)	134	0.346	0.038	0.270	0.038	6.80
XX. Porter . . . . . (5.3% alcohol)	127	0.371	0.036	0.320	0.015	2.19
XXII. Residue of porter (like XX.)	90	0.352	0.014	0.280	0.058	2.29

These results afford tangible evidence of the stimulating action of the liquors examined, as shown in the increased volume of gastric contents, accompanied by increase in acidity. That alcohol is an important factor in the production of these phenomena seems certain. Contrast, for example, Experiment XX. with XXII., which differs only in the absence of the alcohol. But the wines and malted beverages contain a variety of other constituents such as organic acids,<sup>1</sup> which perhaps contribute to increase the stimulating effect, and are doubtless partly responsible in a number of experiments for the high acidity observed. The contrast between the action of water and wine is strikingly shown in Experiments XVI. *a* and *β* carried out on the same animal.

<sup>1</sup> Cf. CHITTENDEN and MENDEL: *loc. cit.*, pp. 56, 80.

COMPARISON OF THE TWO EXPERIMENTS (XVI.  $\alpha$ ,  $\beta$ ).

Fluid introduced in stomach.	Fluid removed from stomach after 3 hours.	Relative volume. per cent.	Total acidity.	Free HCl.	Loosely combined HCl.	Salts.	Total solids.
135 c.c. water	110 c.c.	81	0.274	0.241	0.018	0.015	0.77
135 c.c. white wine }	170 c.c.	126	0.425	0.342	0.018	0.065	1.79

The marked increase in total solids in many of these experiments, however, is not to be attributed, as it is in the case of pure alcohol, entirely to the increased secretion; it is rather in part accounted for by the unabsorbed constituents of the liquor employed. The following table, compiled from analyses at hand, shows that a large portion of the total solids in the gastric juices obtained may be derived from other sources than the secretion itself: —

TABLE SHOWING TOTAL SOLIDS OF GASTRIC CONTENTS.

Nature of fluid introduced into stomach.	Total solids introduced into stomach.	Total solids in gastric contents at end of experiment.
II. Water . . . . .	0 grams.	0.84 grams.
IX. Weak alcohol . .	0 “	0.69 “
VIII. Strong alcohol . .	0 “	4.50 “
XIV. Whiskey . . . .	0.15 “	1.34 “
XVI. White wine . . .	2.8 “	2.41 “
XVII. Claret . . . . .	3.9 “	4.28 “
XIII. Sherry . . . . .	2.35 “	2.78 “
XVIII. Beer . . . . .	7.0 “	10.00 “
XXIII. Beer residue . .	9.1 “	11.56 “
XX. Porter . . . . .	6.6 “	4.16 “
XXI. Porter residue . .	6.6 “	3.10 “

E. Character of the Gastric Juice obtained by Stimulation with Alcohol. — The gastric juice obtained as a result of the stimulating influence of alcohol and alcoholic liquors resembles that ordinarily

procured from gastric fistulæ in its physical characters; it is a thin, colorless, or very faintly yellow fluid containing occasional flocks of mucus in suspension. There was no evidence of irritation or hyperæmia of the mucosa, and all traces of blood were absent. After the doses used, the gastric lining was of a pale or faintly pink color when removed after bleeding the animal. When colored alcoholic liquors were employed, the gastric contents retained the characteristic coloring matter, the latter not being absorbed, while the alcohol entirely disappeared. In chemical composition, the gastric juice appeared somewhat more acid than that ordinarily secreted. It likewise contained a larger amount of solid matter, and in harmony with this fact the proportion of combined hydrochloric acid was increased, which in turn suggests the presence of a somewhat larger amount of proteid or other like matter. The fluids were repeatedly tested with boiled fibrin for proteolytic action, and this was always found vigorous. In the experiments in which alcohol was introduced directly into the intestine (Experiments XI., XII., p. 182), the intestinal lining was not abnormal in appearance, the reaction being alkaline to litmus in the upper duodenum and neutral or faintly alkaline further along the alimentary canal. This corresponds with the observations on the normal reaction of the intestinal contents of the dog, by Moore and Rockwood,<sup>1</sup> whose statements we have repeatedly verified.

#### GASTRIC DIGESTION.

Since chemical, mechanical, and physiological processes go on side by side during digestion, we have carried out a series of experiments to determine in what way and to what extent the factors already investigated combine or coöperate under the influence of alcohol and alcoholic liquors. Our method has included the examination of the stomach contents after test meals were given. The statements current in the literature on this subject are by no means concordant.

In experiments on a woman having a gastric fistula Kretschy<sup>2</sup> observed that alcohol retarded digestion. Buchner<sup>3</sup> found that in

<sup>1</sup> MOORE and ROCKWOOD: *Journal of physiology*, 1897, xxi, p. 373.

<sup>2</sup> KRETSCHY: *Deutsches Arch. f. klin. Med.*, xviii, p. 527; *Jahresbericht f. Thierchemie*, 1876, vi, p. 173.

<sup>3</sup> BUCHNER: *Deutsches Arch. f. klin. Med.*, xxix, p. 537; *Jahresbericht f. Thierchemie*, 1881, xi, p. 286.

the human stomach alcohol, wine, and beer all retarded digestion, though not so markedly as in artificial digestion. Bikfalvi,<sup>1</sup> in observations on dogs, obtained a retardation of digestion with even small quantities of alcohol. Beer and wine showed no favorable influence, the latter even retarding digestion when given in large quantities. Ogáta<sup>2</sup> states that beer, wine, and brandy retard gastric digestion noticeably. Schelhaas<sup>3</sup> observed that in the living stomach wine did not retard digestion so long as there was free HCl present; pathological conditions (carcinoma ventriculi) formed the only exceptions. In an extensive series of experiments, Gluzinski<sup>4</sup> distinguishes two phases occurring during digestion in the stomach in the presence of alcohol, (1) a retardation of proteid digestion, and (2) secretion of a very active, strongly acid gastric juice. Henczinski<sup>5</sup> found no bad effect on digestion following the use of beer. Blumenau<sup>6</sup> states that 25-50 per cent alcohol introduced into the healthy stomach induces a decrease in digestive action during the first two or three hours. Wolffhardt,<sup>7</sup> experimenting on a healthy man, concluded that 15-20 grams of absolute alcohol interfere with proteid digestion, while the effect of cognac varies with the period of digestion during which it is taken; he found that wines tend to promote digestion.

With reference to the motor functions of the stomach Lauder Brunton states that alcohol taken into this organ increases its movements as well as its secretory activity, and by mixing its contents more thoroughly with the gastric juice accelerates digestion.<sup>8</sup> Likewise Klemperer<sup>9</sup> states as a result of his experiments that the motor

<sup>1</sup> BIKFALVI: Jahresbericht f. Thierchemie, 1885, xv, p. 273.

<sup>2</sup> OGÁTA: Jahresbericht f. Thierchemie, 1885, xv, p. 274; Arch. f. Hygiene, 1885, iii, p. 204.

<sup>3</sup> SCHELHAAS: Deutsches Arch. f. klin. Med., xxxvi, p. 427; Jahresbericht f. Thierchemie, 1885, xv, p. 271.

<sup>4</sup> GLUZINSKI: Deutsches Arch. f. klin. Med., 1886, xxxix, p. 405; Jahresbericht f. Thierchemie, 1886, xvi, p. 263.

<sup>5</sup> HENCZINSKI: Dissertation, 1886. Quoted by MUNK: Die Ernährung, p. 327.

<sup>6</sup> BLUMENAU: Therapeutische Monatshefte, 1890, v, p. 504; Jahresbericht f. Thierchemie, 1891, xxi, p. 212.

<sup>7</sup> WOLFFHARDT: Münchn. med. Wochenschr., 1890, xxxvii, p. 608; Centralbl. f. med. Wissen., 1891, p. 47.

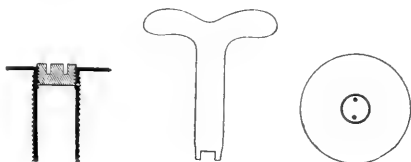
<sup>8</sup> BRUNTON: Disorders of digestion, 1886, p. 146.

<sup>9</sup> KLEMPERER: Zeitschr. f. klin. Med., 1890, xvii, Supp., p. 324; Centralbl. f. med. Wissen., 1891, p. 751.

functions are decidedly increased, as measured by the oil method, while Haan<sup>1</sup> has more recently advanced similar conclusions as the result of work by another method. Gluzinski,<sup>2</sup> however, notes that alcohol diminishes the mechanical action of the stomach in moderate degree.

In considering the selection of subjects for experiment in the direction indicated, preference has been given to dogs. The series of investigations on man above referred to are already extensive, and the difficulties of obtaining definite answers to specific questions by this method of experimentation are obvious. It is rarely possible or desirable to carry out a large number of determinations on any single individual, while it is likewise practically impossible to control the physiological condition of the individual, *i. e.*, diet, etc., over prolonged periods. The animals used in this research were large dogs of 21 and 25 kilos; gastric fistulæ were made, and a German silver cannula introduced into the fundus of

the stomach. In place of a cork, metal stoppers were devised to screw into the inner cannula tube by means of a small metallic key. The arrangement is shown in the dia-



gram. The wounds healed perfectly, and the animals remained in good health during the entire period of investigation, covering several months. Irregularities of diet were avoided by feeding definite portions of prepared dog biscuit with water; this food was eagerly eaten, and sufficed to keep the dogs in physiological equilibrium.

The determinations of the acidity of the stomach contents were carried out according to the method of Töpfer.<sup>3</sup> The gastric fluid was occasionally centrifugalized when food particles prevented pipetting off the fluid portion. Where only small quantities of fluid were available the titrations with phenolphthaliën and dimethylamidoazobenzol were combined in the same 5 c.c. of fluid according to the recommendation of Einhorn.<sup>4</sup> Comparative experiments show that this modification gives the same values as the original method. Thus in one experiment: —

<sup>1</sup> HAAN: *Comptes rendus de la société de biologie*, 1895, ii, p. 816.

<sup>2</sup> GLUZINSKI: *loc. cit.*

<sup>3</sup> TÖPFER: *Zeitschr. f. physiol. Chemie*, 1894, xix, p. 104.

<sup>4</sup> EINHORN: *New York medical journal*, 1896, May 9, p. 603.

	Total acidity with <i>Phenolphthaleïn.</i>	Free HCl with <i>Dimethylamidoazobenzol.</i>
Töpfer method . . . . . (separate titrations)	$\left\{ \begin{array}{l} 1.55 \text{ c.c. } \frac{N}{10} \text{NaOH} \\ = 0.112 \text{ per cent HCl.} \end{array} \right.$	$\left\{ \begin{array}{l} 1.0 \text{ c.c. } \frac{N}{10} \text{NaOH} \\ = 0.072 \text{ per cent HCl.} \end{array} \right.$
Einhorn-Töpfer method . . . (combined titration)	$\left\{ \begin{array}{l} 1.55 \text{ c.c. } \frac{N}{10} \text{NaOH} \\ = 0.112 \text{ per cent HCl.} \end{array} \right.$	$\left\{ \begin{array}{l} 1.0 \text{ c.c. } \frac{N}{10} \text{NaOH} \\ = 0.072 \text{ per cent HCl.} \end{array} \right.$

Our experience with Töpfer's method (or Einhorn's modification) leads us to agree with P. Häri<sup>1</sup> that in the absence of free HCl, *i. e.*, when no reaction is obtained with the dimethylamidoazobenzol reagent, the quantitative determinations of HCl by this method cease to be accurate, and under such conditions it cannot be employed. The occurrence of such conditions, however, is not frequent in the dog; we have observed the absence of free HCl (during digestion) in one animal under circumstances resembling those of acute gastric catarrh.<sup>2</sup> The food—dog biscuit—was largely undigested many hours after the meal, the acidity was high (0.55–0.594 per cent expressed as HCl), and the gastric contents possessed an odor strongly suggesting fatty acids. Lactic acid was found present (Uffelmann's test).

In view of the increased volume of fluid found in the stomach when alcohol is introduced into that organ after ligation of the pylorus, it was of interest to learn what results follow under normal conditions of the pylorus. For this purpose 20 to 25 per cent alcohol, slightly warmed, was introduced through the gastric cannula, and at the end of 30 minutes the gastric contents were discharged into a graduated vessel. Control experiments were made with distilled water, both fluids always being introduced into the empty stomach. This condition of the organ is shown by the lack of spontaneous flow when the cannula is opened, as well as by absence of free HCl. Flocks of mucus, alkaline to litmus, are usually present. The data obtained show no marked agreement, the fluid as a rule

<sup>1</sup> HÄRI, P.: Arch. f. Verdauungskrankh., ii, pp. 182, 332; Centralbl. f. Physiologie, 1896, x, p. 731.

<sup>2</sup> Cf. v. JAKSCH: Klinische Diagnostik innerer Krankheiten, 4te Auflage, p. 200.



rapidly disappearing from the stomach. In 17 experiments with water, the *average* relative volume recovered from the stomach through the cannula at the end of the thirty minutes after introduction of quantities from 40–200 c.c. was about 30 per cent. Fourteen similar experiments with alcohol gave an average of 45 per cent. It is natural to ascribe the relatively greater volumes found in the stomach after the use of alcohol to an increased secretion of gastric juice occurring along with the rapid expulsion of fluid through the pylorus, and not to a retardation of the motor functions; for current statements assume increased motility of the stomach under the influence of alcohol,<sup>1</sup> while the experiments already reported justify the explanation given. Much emphasis cannot, however, be placed upon the averages given above, since the individual results vary widely among themselves, and no constant corresponding variations in acidity were observed, as in the experiments with ligated pylorus.

In the following series of experiments test meals were given, and the influence of alcohol and a considerable number of alcoholic beverages contrasted with that of water. Attention was directed to (1) variations in acidity and (2) time of digestion. Fifty grams of finely chopped lean meat were fed to the dog in each experiment, the stomach having been previously examined and found empty. Meat was chosen for the test meal because experience in this laboratory has shown that its composition, when it is obtained as described, does not vary much from time to time; and after a trial of mixed food, *e. g.* dog biscuit, it seemed more satisfactory to employ a simple diet in which proteid preponderated. Similar recommendation is made by v. Jaksch in considering test meals for the human subject.<sup>2</sup> Alcoholic fluids or water were introduced slightly warmed<sup>3</sup> into the stomach through the fistula, since dogs usually refuse to take the former by way of the mouth. At definite intervals of one-quarter to one-half hour, small quantities of gastric contents were permitted to flow out of the fistula. Total acidity (expressed as HCl), free and loosely combined HCl were determined by the method already described. The process of digestion in the stomach lasted, under the conditions described, about three hours, the average duration varying

<sup>1</sup> Cf. references p. 190.

<sup>2</sup> v. JAKSCH: *loc. cit.*, p. 192.

<sup>3</sup> Cf. note 4, p. 176.

somewhat with the animal.<sup>1</sup> There was no very gradual diminution of undissolved meat particles noticeable until toward the end of this period, when the stomach very soon became empty. This corresponds with the observations of Kühne on man and the dog, in experiments with duodenal fistulæ.<sup>2</sup> This investigator found only a slight disappearance of contents from the stomach until near the end of the digestion period, when the great bulk of material, excepting larger pieces of food, was discharged at once through the pylorus. Richet arrived at similar conclusions in experiments on man.<sup>3</sup> We have usually observed a complete emptying of the stomach within a period of thirty minutes; the conclusion of this process is designated in the notes as the "end of gastric digestion." Protocols of experiments follow.

#### ANALYSES OF ALCOHOLIC BEVERAGES USED.

	Alcohol by vol. per cent.	Dry solids. per cent.		Alcohol by vol. per cent.	Dry solids. per cent.
Gin . . . .	51.0	0.29	Stout . . . .	6.2	5.4
Whiskey . .	50.0	0.32	Claret . . . .	5.2	3.2
Sherry . . .	21.75	4.7	Porter . . . .	5.3	4.4
White Wine .	13.32	2.5	Beer . . . .	4-5	7.0

#### DOG A. — Weight 25 kilos.

##### I. 9.25 A.M. 50 grams meat (no water).

	ANALYSIS OF CONTENTS		
	Total acidity.	Loosely combined HCl.	Free HCl.
9.55	0.382	0.292	0.104
10.35	0.425	0.234	0.148
11.10	0.425	0.220	0.180
11.45	0.407	0.224	0.176
12.15	Stomach empty; end of gastric digestion.		
	Time of digestion = 2 hours and 55 minutes.		

<sup>1</sup> In experiments on a man, with a similar meal, Jessen found the digestion time equalled 2 to 3 hours. *Zeitschr. f. Biologie*, 1883, xix, p. 149.

<sup>2</sup> KÜHNE: *Lehrbuch der physiol. Chemie*, 1868, p. 53.

<sup>3</sup> RICHEL: Quoted in GAMGEE: *Physiological chemistry*, 1893, ii, p. 159.

**II. 9.10 A.M. 50 grams meat + 50 c.c. water.**

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.30	0.241	0.144	0.093
10.00	0.295	0.169	0.108
10.20	0.367	0.216	0.115
10.40	0.439	0.288	0.144
11.30	Stomach empty; end of gastric digestion.		

Time of digestion = 2 hours and 20 minutes.

4.

**III. 9.30 A.M. 50 grams meat + 100 c.c. water.**

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
10.00	0.299	0.173	0.090
10.30	0.475	0.230	0.122
11.00	0.518	0.230	0.173
11.15	0.497	0.202	0.241
11.35	0.494	0.191	0.202
11.50	0.479	0.205	0.195
12.10	0.382	0.194	0.187
12.30	Stomach empty; end of gastric digestion.		

Time of digestion = 3 hours.

**IV. 2.10 P.M. 50 grams meat + 150 c.c. water.**

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
2.40	0.252	0.137	0.108
3.10	0.374	0.194	0.130
3.40	0.533	0.245	0.198
3.55	0.547	0.234	0.234
4.10	0.490	0.205	0.216
4.25	0.385	...	0.101
4.40	Stomach empty; end of gastric digestion.		

Time of digestion = 2 hours and 30 minutes.

**V. 9.05 A.M. 50 grams meat + 150 c.c. carbonated water.**

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.35	0.263	0.083	0.122
10.05	0.360	0.158	0.140
10.35	0.468	0.194	0.216
10.50	0.486	0.205	0.216
11.05	0.540	0.234	0.198
11.25	0.580	0.234	0.248
11.45	Stomach empty; end of gastric digestion.		

Time of digestion = 2 hours and 40 minutes.

## VI. 1.00 P.M. 50 grams meat + 100 c.c. 10 per cent alcohol.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
2.45	0.497	0.209	0.230
3.10	0.464	0.220	0.173
3.30	0.436	0.180	0.202
3.50	0.400	0.162	0.202
4.10	0.263	. . .	0.094
4.30	Stomach empty; end of gastric digestion.		
Time of digestion = 3 hours and 30 minutes.			

## VII. 2.30 P.M. 50 grams meat + 50 c.c. 20 per cent alcohol.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
3.00	0.313	0.118	0.090
3.30	0.374	0.187	0.176
4.00	0.439	0.194	0.151
4.30	0.515	0.205	0.184
5.00	0.407	0.144	0.248
5.30	0.264	....	0.155
5.30	Stomach nearly empty; end of gastric digestion.		
Time of digestion = <b>3 hours.</b>			

## VIII. 12.45 P.M. 50 grams meat + 50 c.c. 20 per cent alcohol.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
2.30	0.439	0.213	0.158
2.50	0.457	0.191	0.205
3.10	0.493	0.205	0.227
3.30	0.364	0.129	0.187
3.50	Stomach practically empty; end of gastric digestion.		
Time of digestion = <b>3 hours and 5 minutes.</b>			

## IX. 9.15 A.M. 50 grams meat + 50 c.c. 30 per cent alcohol.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.40	0.191	0.130	0.058
10.05	0.335	0.155	0.151
10.30	0.421	0.176	0.180
10.50	0.468	0.184	0.201
11.10	0.460	0.165	0.220
11.30	0.410	0.148	0.220
11.50	0.468	0.195	0.244
12.10	0.417	0.112	0.240
12.30	0.360	0.086	0.216
1.00	Stomach empty; end of gastric digestion.		
	Time of digestion = <b>3 hours and 45 minutes.</b>		

**X.** 9.00 A.M. 50 grams meat + 150 c.c. **Hochheimer.**

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.30	0.374	0.140	0.176
10.00	0.432	0.154	0.191
10.15	0.450	0.151	0.198
10.45	0.497	0.187	0.220
11.15	0.533	0.198	0.271
11.30	0.555	0.241	0.227
12.00	0.508	0.248	0.173
12.15	Stomach empty; end of gastric digestion.		
	Time of digestion = 3 hours and 15 minutes.		

**XI.** 9.00 A.M. 50 grams meat + 50 c.c. **whiskey** + 50 c.c. **water.**

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.30	0.252	0.101	0.119
10.00	0.392	0.176	0.176
10.30	0.403	0.151	0.191
11.00	Stomach empty; end of gastric digestion.		
	Time of digestion = 2 hours.		

**XII.** 2.45 P.M. 50 grams meat + 50 c.c. **whiskey** + 50 c.c. **water.**

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
3.15	0.230	0.076	0.119
3.45	0.320	0.097	0.220
4.15	0.468	0.198	0.212
4.30	0.508	0.198	0.198
4.45	0.490	0.184	0.212
5.15	0.569	0.205	0.252
5.45	Stomach empty; end of gastric digestion.		
	Time of digestion = 3 hours.		

**XIII.** 1.00 P.M. 50 grams meat + 50 c.c. **gin** + 25 c.c. **water.**

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
2.00	0.439	0.173	0.194
2.30	0.450	0.170	0.197
2.45	0.428	0.158	0.238
3.00	0.442	0.154	0.212
3.15	0.410	0.140	0.215
3.30	0.420	0.143	0.234
3.45	0.338	0.122	0.180
4.00	Stomach empty; end of gastric digestion.		
	Time of digestion = 3 hours.		

**XIV.** 9.20 A.M. 50 grams meat + 50 c.c. brandy + 25 c.c. water.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.50	0.237	0.159	0.065
10.20	0.368	0.201	0.133
10.50	0.465	0.230	0.205
11.20	0.533	0.267	0.194
11.40	0.468	....	0.158
12.00	Stomach empty; end of gastric digestion.		
Time of digestion = 2 hours and 40 minutes.			

**XV.** 2.50 P.M. 50 grams meat + 150 c.c. lager beer.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
3.20	0.259	0.112	0.115
3.50	0.410	0.205	0.148
4.20	0.518	0.245	0.184
4.35	0.572	0.248	0.230
4.50	0.569	0.252	0.208
5.05	0.547	0.220	0.238
5.20	0.508	0.162	0.211
5.35	0.475	0.162	0.238
5.50	0.413	0.115	0.241
6.05	Stomach empty; end of gastric digestion.		
Time of digestion = <b>3 hours and 15 minutes.</b>			

**XVI.** 9.40 A.M. 50 grams meat + 150 c.c. stout.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
10.10	0.364	0.140	0.187
10.40	0.446	0.166	0.180
11.10	0.555	0.220	0.295
11.40	0.616	0.212	0.302
12.10	0.580	0.266	0.247
12.40	Stomach empty; end of gastric digestion.		
Time of digestion= <b>3 hours.</b>			

**XVII a.** 9.15 A.M. 50 grams meat + 150 c.c. beer.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.45	0.248	0.151	0.082
10.15	0.367	0.201	0.123
10.45	0.457	0.238	0.137
11.20	0.526	0.266	0.209
11.40	0.511	0.213	0.223
12.15	0.465	0.216	0.176
12.30	Stomach empty; end of gastric digestion.		
Time of digestion = <b>3 hours and 15 minutes.</b>			

**XVII β.** 3.00 P.M. 50 grams meat + 150 c.c. water.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
3.30	0.227	0.130	0.090
4.00	0.400	0.209	0.129
4.30	0.522	0.274	0.158
5.00	0.583	0.310	0.195
5.15	0.583	0.302	0.205
5.30	0.446	0.209	0.184
5.45	0.569	0.298	0.127
6.00	Stomach empty; end of gastric digestion.		
	Time of digestion = 3 hours.		

**XVIII α.** 8.30 A.M. 50 grams meat + 50 c.c. water.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.00	0.371	0.227	0.126
9.30	0.443	0.274	0.144
10.00	0.518	0.252	0.234
10.30	0.569	0.263	0.252
11.00	Stomach empty; end of gastric digestion.		
	Time of digestion = 2 hours and 30 minutes.		

**XVIII β.** 2.10 P.M. 50 grams meat + 100 c.c. 30 per cent alcohol.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
2.40	0.234	0.112	0.101
3.10	0.352	0.165	0.137
3.40	0.490	0.209	0.162
4.10	0.550	0.263	0.191
4.40	0.550	0.245	0.201
5.10	Stomach empty; end of gastric digestion.		
	Time of digestion = 3 hours.		

**XIX α.** 9.00 A.M. 50 grams meat + 100 c.c. water.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.30	0.324	0.165	0.137
10.00	0.378	0.198	0.144
10.30	0.494	0.259	0.169
11.00	0.487	0.220	0.188
11.15	0.457	0.205	0.131
11.30	Stomach empty; end of gastric digestion.		
	Time of digestion = 2 hours and 30 minutes.		

**XIX**  $\beta$ . 2.30 P.M. 50 grams meat + 150 c.c. lager beer.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
3.00	0.260	0.119	0.137
3.30	0.378	0.201	0.137
4.00	0.465	0.191	0.188
4.30	0.533	0.223	0.248
4.45	0.562	0.233	0.306
5.10	0.465	0.223	0.176
5.30	Stomach empty; end of gastric digestion.		
	Time of digestion = 3 hours.		

**XX**  $\alpha$ . 9.15 A.M. 50 grams meat + 75 c.c. sherry + 25 c.c. water.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.45	0.295	0.108	0.155
10.15	0.331	0.101	0.173
10.45	0.367	0.133	0.187
11.15	0.418	0.158	0.212
11.30	0.436	0.169	0.216
11.45	0.490	0.191	0.248
12.00	Stomach empty; end of gastric digestion.		
	Time of digestion = 2 hours and 45 minutes.		

**XX**  $\beta$ . 2.30 P.M. 50 grams meat + 150 c.c. carbonated water.

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
3.00	0.238	0.043	0.126
3.30	0.360	0.130	0.176
4.00	0.432	0.187	0.169
4.30	0.533	....	0.169
4.45	Stomach empty; end of gastric digestion.		
	Time of digestion = 2 hours and 15 minutes.		

**Dog B. — Weight 21 kilos.****I.** 1.45 P.M. 50 grams meat (no water).

ANALYSIS OF CONTENTS			
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
2.15	0.353	0.191	0.118
2.40	0.443	0.222	0.180
3.00	0.511	0.227	0.198
3.20	0.525	0.227	0.280
3.45	0.572	0.260	0.209
4.15	0.568	0.349	0.195
4.45	Stomach empty; end of gastric digestion.		
	Time of digestion = 3 hours.		



# *Influence of Alcoholic Drinks upon Digestion.*

201

## II. 9.15 A.M. 50 grams meat + 50 c.c. water.

	ANALYSIS OF CONTENTS		
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.50	0.302	0.220	0.082
10.15	0.432	0.223	0.144
10.45	0.472	0.201	0.252
11.15	0.472	0.144	0.288
11.35	0.484	0.155	0.270
11.55	0.453	0.144	0.306
12.15	0.407	0.100	0.241
12.30	0.400	0.133	0.234
12.45	0.306	....	0.216

End of gastric digestion.

Time of digestion = 3 hours and 30 minutes.

## III. 9.15 A.M. 50 grams meat + 50 c.c. 20 per cent alcohol + water.

	ANALYSIS OF CONTENTS		
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.50	0.136	0.086	0.036
10.15	0.285	0.108	0.144
10.45	0.479	0.173	0.244
11.15	0.472	0.177	0.252
11.35	0.518	0.237	0.252
11.55	0.486	....	0.209
12.15	0.421	....	0.213

12.30 Stomach empty; end of gastric digestion.

Time of digestion = 3 hours and 15 minutes.

## IV. 8.50 A.M. 50 grams meat + 100 c.c. 30 per cent alcohol.

	ANALYSIS OF CONTENTS		
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.20	0.324	....	0.144
9.50	0.493	....	0.072
10.20	0.641	....	0.100
10.50	0.547	0.338	0.166
11.20	0.588	....	0.206
11.50	0.544	....	0.230
12.20	....	....	present.

12.30 End of gastric digestion.

Time of digestion = 3 hours and 40 minutes.

## V. 2.45 P.M. 50 grams meat + 75 c.c. claret.

	ANALYSIS OF CONTENTS		
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
3.15	0.396	0.155	0.216
3.45	0.450	0.238	0.158
4.15	0.576	....	0.209

4.45 End of gastric digestion.

Time of digestion = 2 hours.

VI $\alpha$ . 9.15 A.M. 50 grams meat + 150 c.c. beer.

	ANALYSIS OF CONTENTS		
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
9.45	0.273	0.144	0.104
10.15	0.367	0.187	0.155
10.45	0.464	0.223	0.194
11.15	0.616	0.345	0.256
11.45	0.501	0.238	0.170
12.15	0.508	....	0.151
12.30	0.533	....	0.187
12.45	0.468	...	0.158
1.00	End of gastric digestion.		
	Time of digestion = 3 hours and 45 minutes.		

VI $\beta$ . 1.00 P.M. 50 grams meat + 150 c.c. water.

	ANALYSIS OF CONTENTS		
	<i>Total acidity.</i>	<i>Loosely combined HCl.</i>	<i>Free HCl.</i>
2.00	0.620	0.282	0.201
2.30	0.590	0.266	0.234
3.00	0.666	0.392	0.224
3.30	0.627	....	0.206
4.00	...	....	trace.
4.15	End of gastric digestion.		
	Time of digestion = 3 hours and 15 minutes.		

In the accompanying table the "time of digestion" of the experiments preceding is given in hours. The experiments marked *a* and  *$\beta$*  are strictly comparable, as reference to the protocols will show that they were carried out in succession on the same day.

From these results it is apparent that the time of digestion in the stomach for the proteid test meal employed is not greatly varied under the influence of alcohol. The results obtained suggest, however, a tendency toward prolongation of the period during which the meat remains in the stomach when alcoholic fluids are present. This tendency is most noticeable in the case of Dog A, and particularly in those experiments which immediately succeed each other on the same day and are therefore strictly comparable. The differences are too small, however, to have any great significance. Retardation is perhaps more marked with the malted beverages, and is apparently out of proportion to the alcohol present. With reference to the changes in the acidity of the stomach contents a large number of observations disclose no specific differences in the various digestions. The varia-

TABLE OF TIME OF DIGESTION (IN HOURS).

Dog A.					Dog B.			
No.	Water.	Alcohol.	Weak alcoholic beverages.	Strong alcoholic beverages.	No.	Water.	Alcohol.	Weak alcoholic beverages.
	h. m.	h. m.	h. m.	h. m.		h. m.	h. m.	h. m.
XVII $\alpha$ {	...	...	3 15	...	I	3	...	...
XVII $\beta$ }	3	...	...	...	II	3 30	...	...
I	2 55	...	...	...	III	...	3 15	...
II	2 20	...	...	...	IV	...	3 40	...
VII	...	3	...	...	V	...	...	2
VI	...	3 30	...	...	VI $\alpha$ {	...	...	3 45
VIII	...	3 05	...	...	VI $\beta$ }	3 15	...	...
IX	...	3 45	...	...		...	...	...
XVIII $\alpha$ {	2 30	...	...	...		...	...	...
XVIII $\beta$ }	...	3	...	...		...	...	...
XIV	...	...	...	2 40		...	...	...
XV	...	...	3 15	...		...	...	...
XIX $\alpha$ {	2 30	...	...	...		...	...	...
XIX $\beta$ }	...	...	3	...		...	...	...
XVI	...	...	3	...		...	...	...
IV	2 30	...	...	...		...	...	...
X	...	...	3 15	...		...	...	...
III	3	...	...	...		...	...	...
XIII	...	...	...	3		...	...	...
XX $\alpha$ {	...	...	...	2 45		...	...	...
XX $\beta$ }	2 15	...	...	...		...	...	...
V	2 40	...	...	...		...	...	...
XI	...	...	...	2		...	...	...
XII	...	...	...	3		...	...	...
Average.	2 40	3 20	3 10	2 40		3 15	3 30	2 52

tions are common to all the experiments. They include a gradual rise in total acidity during approximately the first two hours of diges-

tion, followed by a gradual decrease until the stomach becomes empty; at this point free HCl is absent. The combined HCl increases with the progress of digestion, the products of proteolysis combining with relatively larger quantities of free acid.<sup>1</sup> Since the secretion of acid is continually progressing in the stomach, the percentage of free HCl increases gradually in the course of the digestion, likewise decreasing rapidly toward the end of this process. In agreement with our previous statements relative to the rather sudden discharge of the gastric contents into the intestine (p. 194), an abrupt decline in acidity toward the end of the digestion period was frequently observed. Evidence of an "after period" of secretion was not obtained.<sup>2</sup>

#### DISAPPEARANCE OF ALCOHOL FROM THE STOMACH.

It has long been known that alcohol disappears rapidly from the alimentary canal, and even so early as 1847 Bouchardat and Sandras stated that the absorption takes place from the stomach especially.<sup>3</sup> More recent and conclusive experiments in which the pylorus has been artificially closed, have demonstrated with certainty that alcohol, in distinction from water, is readily absorbed from the stomach.<sup>4</sup> Furthermore, many substances like sugar, peptone, etc., are readily absorbed from the stomach in the presence of alcohol, while their absorption from the intestine is likewise accelerated by this substance.<sup>5</sup> Thus an ordinary dose of chloral hydrate introduced in watery solution into a stomach with ligated pylorus fails to bring about narcosis;<sup>6</sup> if, however, a quantity of alcohol too small of itself to produce any pharmacological action be present, narcosis follows, just as when the open pylorus permits the intestine to participate in the absorption.

The complete disappearance of alcohol from the stomach has been observed by us in a large number of experiments in which the pylorus

<sup>1</sup> Cf. CHITTENDEN: Digestive proteolysis, 1894, pp. 53 *seq.*

<sup>2</sup> Cf. GLUZINSKI: Jahresbericht f. Thierchemie, 1886, xvi, p. 264.

<sup>3</sup> BOUCHARDAT and SANDRAS: Annales de chimie et de physique, 1847, xxi, 3 Série, p. 456.

<sup>4</sup> Cf. for example, TAPPEINER: Zeitschr. f. Biologie, 1881, xvi, p. 497; BRANDL: *ibid.*, 1892, xxix, p. 277; v. MERING: Jahresbericht f. Thierchemie, 1893, xxiii, p. 293.

<sup>5</sup> Cf. for example, J. v. SCANZONI: Zeitschr. f. Biologie, 1896, xxxiii, p. 462.

<sup>6</sup> Cf. also experiments with strychnine. MELTZER: Journ. of exper. medicine, 1896, i, p. 529.

was ligated. The following results tabulated from the experiments on secretion (pp. 179-186), demonstrate this statement: —

TABLE SHOWING ABSORPTION OF ALCOHOL FROM STOMACH.

No.	Weight of dog. Kilos.	Duration of experiment.		Volume of fluid introduced. ccm.	Content of alcohol. Per cent by vol.	Alcohol found at end of experiment. grams.
		h.	m.			
VII	23.0	3	30	200 (alcohol)	37.5	4
VIII	21.0	3	00	200 ( " )	37.5	4-5
IX	8.0	3	50	100 ( " )	5.0	0
X	7.3	3	45	110 ( " )	4.8	0
XIII	10.7	3	55	75 (sherry)	21.0	0
XIV	18.5	3	45	150 (whiskey)	16.0	0
XV	8.0	3	45	125 (wine)	13.3	0
XVI	25.0	3	00	135 ( " )	13.3	0
XVII	12.3	4	00	125 (claret)	5.15	0
XVIII	10.2	3	55	100 (beer)	4.5	0
XX	14.0	3	45	150 (porter)	3.75	0
XXI	8.5	3	55	125 (beer)	4.7	0

The rapid discharge of watery or alcoholic fluids from the stomach through the pylorus has already been referred to on p. 193. The results are in harmony with those obtained by v. Mering on dogs with duodenal fistulæ.<sup>1</sup> In his experiments, for example, 500 c.c. being administered to a large dog, 490 c.c. were expelled through the pylorus in twenty minutes. The rapidity of expulsion was found to depend on the state of repletion of the small intestine, — an observation in accord with the retarded evacuation of the stomach seen when food is given along with fluids. v. Mering further observed that when water holding CO<sub>2</sub> in solution enters the stomach, the gas is readily absorbed; <sup>2</sup> alcohol is likewise absorbed, as J. Miller has recently verified for the human stomach.<sup>3</sup> Ogáta<sup>4</sup> found that of 6.5-8.8 grams

<sup>1</sup> v. MERING: Quoted in GAMGEE: *Physiological chemistry*, 1893, ii, pp. 441 *seq.*

<sup>2</sup> Cf. also Experiment V., p. 178.

<sup>3</sup> MILLER, J.: *Arch. f. Verdauungskrankh.*, i, p. 233. *Jahresbericht f. Thierchemie*, 1895, xxv, p. 293.

<sup>4</sup> OGÁTA: *Jahresbericht f. Thierchemie*, 1885, xv, p. 274.

of alcohol introduced into the stomach in wine or beer, 80-90 per cent disappeared within half an hour. In the presence of soluble products in the stomach, an excretion of water by that organ is said to result in proportion to the amount of substance absorbed, — an idea akin to the one suggested in explanation of the relatively larger quantities of fluid found in the unligated stomach soon after introduction of alcohol, as compared with water. The experiments which we have made verify the statements of the investigators mentioned, as the following data selected from protocols indicate: —

*Data showing disappearance of alcohol from unligated stomach.*

I. Dog, with gastric fistula.

- a. 3.45 p. m. Introduced 50 c.c. 20 per cent alcohol into stomach.
- 4.15 “ Removed gastric contents = 40 c.c. No alcohol found.
- b. 3.15 “ Introduced 40 c.c. 25 per cent alcohol.
- 3.45 “ Removed gastric contents = 20 c.c. No alcohol found.
- c. 2.40 “ Introduced 125 c.c. 20 per cent alcohol.
- 3.10 “ Removed a portion of gastric contents. Free HCl = 0.072 per cent. Small amount of alcohol present.

II. Dog of 18 kilos, employed in a salivary experiment. In the course of the latter the animal received at intervals 45 c.c. absolute alcohol diluted with water. Two hours after last portion was given the stomach contents (200 c.c.) were removed. They contained 1.1 grms. alcohol.

III. Dog of 18 kilos. Salivary experiment. At intervals were given 70 c.c. absolute alcohol diluted with water. One and one-third hours after last portion (40 c.c.) was given the stomach contents (350 c.c.) contained 9.4 grms. alcohol.

IV. Dog of 14 kilos. Salivary experiment. 140 c.c. absolute alcohol diluted with water were given in three portions. Three-fourths of an hour after the last portion (50 c.c.), the stomach contents (450 c.c.) contained 24.6 grms. alcohol.

V. Dog of 10 kilos. Salivary experiment. 120 c.c. whiskey, containing 50 per cent of alcohol, were given in two portions. Four and one-half hours after the last portion (60 c.c.) the stomach contents (170 c.c.) contained 2.7 grms. alcohol.

VI. Dog. Salivary experiment. 135 c.c. brandy, containing about 50 per cent of alcohol, were given in two portions. Two hours after last portion (75 c.c.), the stomach contents (240 c.c.) contained 8.8 grms. alcohol.

VII. Dog of 10 kilos. Salivary experiment. 350 c.c. wine containing 5.15 per cent alcohol were given in two portions. One and one-half hours after last portion (200 c.c.), the stomach contents (190 c.c.) contained 5.5 grms. alcohol.

It is of interest to note that the large volumes of fluid (170-450 c.c.) found in the stomach in Experiments II.-VII. correspond with the data already presented with reference to the increased secretion of gastric juice due to alcohol and alcoholic beverages.

#### SUMMARY.

Some of the more important conclusions to be drawn from the results of the experiments reported in the preceding pages may be advantageously summarized here.

Upon the secretion of saliva, the presence of strong alcohol or an alcoholic beverage in the mouth has a direct stimulating effect leading to a sudden increase in the flow of saliva. This acceleration of secretion, however, is of brief duration. The stimulating effect is manifested not only by an increase in the volume of the secretion, but also by an increase in both organic and inorganic constituents. The effect produced is in no sense peculiar to alcohol, but is common to many so-called stimulants, such as dilute acid (vinegar), ether-vapor, etc. Indeed, the effect is precisely analogous to that induced by an increase in intensity of stimulation, when the salivary glands are electrically excited through their nerves.

As to the possibility of alcoholic fluids absorbed from the stomach giving rise to an indirect stimulation of salivary secretion, or exercising any appreciable influence upon the composition of the secretion, our results give a negative answer. Thus, alcoholic fluids introduced directly into the stomach (of dogs) by injection through the stomach wall, thus doing away with any local action in the mouth, produce no appreciable effect upon the rate of secretion, as induced by a constant external stimulus, of either submaxillary or sublingual saliva. Even doses of alcohol sufficient to produce prolonged narcosis when introduced in this way fail to check the flow of saliva. There is likewise no specific influence exerted on the composition of the secretion. Hence, so far as our results go, alcohol and alcoholic fluids are without any specific effect upon the secretion of saliva, except to produce a transitory stimulation of secretion while in the mouth cavity.

Upon gastric secretion, alcohol and alcoholic fluids have a marked effect, increasing very greatly both the flow of gastric juice and also

its content of acid and total solids. Further, this action is exerted not only by the presence of alcoholic fluids in the stomach, but also indirectly through the influence of alcohol absorbed from the intestine. Thus, ordinary ethyl alcohol introduced into the empty stomachs of dogs, with the duodenum ligated, shows a marked stimulating action upon gastric secretion — as compared with the action of water under like conditions — increasing not only the volume of gastric juice very greatly, but also its acidity, content of solid matter, etc. Moreover, alcohol absorbed from the intestine, the latter being entirely shut off from the stomach, may likewise cause stimulation of the gastric glands, with a marked increase in the rate of secretion, etc. Whiskey, brandy, sherry, claret, beer, and porter all agree in producing stimulation of gastric secretion. Further, as already stated, the gastric juice secreted under alcoholic stimulation is more acid, contains more solid matter and more combined hydrochloric acid than the ordinary secretion. It is likewise strongly proteolytic.

If these results are considered in connection with our previous observations upon the influence of alcohol and alcoholic drinks upon the purely chemical processes of gastric digestion; it is seen that side by side with the greater or lesser retardation of digestive proteolysis caused by alcoholic beverages there occurs an increased flow of gastric juice rich in acid and of unquestionable digestive power. The two effects may thus normally counterbalance each other, though it is evident that modifying conditions may readily retard or stimulate the processes in the stomach according to circumstances. Foremost among the latter is the rapid disappearance of alcohol from the alimentary canal.

Since any influence exerted by alcohol or alcoholic beverages upon the solvent or digestive power of the gastric juice in the stomach must depend upon the presence of alcohol in the stomach contents, it follows that the tendency toward rapid removal of the alcohol from the alimentary tract by absorption must necessarily diminish correspondingly the extent of the retardation of gastric digestion which the presence of alcohol in the stomach may occasion. Since, however, the stimulation of gastric secretion induced by alcohol is brought about not only by the direct action of alcohol in the stomach, but also by the indirect action of alcohol absorbed from the intestine, it follows that possible inhibition of the digestive action of the gastric juice would probably be of shorter duration than the stimulation of secretion, and that consequently in the body alcoholic fluids would



hardly lead to any retardation of gastric digestion. This point has been very carefully and thoroughly tested by numerous experiments on healthy dogs with gastric fistulæ, using proteid test meals, with the result that certainly in the stomach of dogs digestion is not retarded in any pronounced degree under the influence of alcohol or alcoholic fluids. Of hastened digestion, the results obtained give little or no suggestion, and we must therefore conclude that the two diverse factors above referred to more or less counterbalance each other so that gastric digestion in the broadest sense of the term is not markedly varied under the influence of alcohol or alcoholic fluids. This conclusion, it may be mentioned, stands in perfect harmony with the results of the investigations of Zuntz and Magnus-Levy regarding the influence of alcohol (beer) on the digestibility and utilization of food in the body. These investigators found by a series of metabolic experiments on men with diets largely made up of milk and bread, and on individuals accustomed and unaccustomed to the use of alcoholic beverages, that the latter did not in any way diminish the utilization of the food by the body.<sup>1</sup>

Especially worthy of note is the rapid disappearance of alcohol from the stomach and alimentary tract when alcoholic fluids are taken. As our results show, the introduction of even 200 c.c. of 37 per cent alcohol into the stomach of a dog with the duodenum ligated at the pylorus may be followed by the nearly complete disappearance of the alcohol in 3-3½ hours by absorption through the stomach walls into the blood. With the outlet from the stomach into the intestine open, the rate of absorption of alcohol is greatly increased. We may well believe, as stated by Ogáta, that when 6-8 grams of alcohol are taken into the stomach in the form of wine or beer that 80-90 per cent of the alcohol will disappear from the alimentary tract inside of half an hour. Indeed, our own experiments on dogs with gastric fistulæ lead to this conclusion. Thus, in one experiment 50 c.c. of 20 per cent alcohol were introduced into the stomach, and on withdrawing the stomach-contents half an hour later no alcohol whatever was found in the 40 c.c. of fluid obtained. In view of this rapid disappearance of alcohol from the alimentary tract it is plain that alcoholic fluids cannot have much, if any, direct influence upon the secretion of either pancreatic or intestinal juice.

<sup>1</sup> ZUNTZ and MAGNUS-LEVY: *Archiv f. d. ges. Physiol.*, 1891, xlix, p. 438; MAGNUS-LEVY: *ibid.*, 1893, liii, p. 544.

# ON THE SIMILARITY OF STRUCTURAL CHANGES PRODUCED BY LACK OF OXYGEN AND CERTAIN POISONS.

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WE know through the experiments of Spallanzani, Pflüger,<sup>1</sup> Bunge,<sup>2</sup> etc., that life phenomena may persist for a comparatively long period in the absence of oxygen, and that at the same time, as has been shown by Liebig and Hermann, a considerable amount of work may be done; but in the face of these facts we are at a loss to understand those cases in which asphyxia so quickly results in death.

Loeb<sup>3</sup> has suggested that when a function fails through lack of oxygen, the failure may in some cases be due to an alteration of the molecular conditions upon which depends the conversion of chemical energy into the particular form of energy set free in the physiological process concerned. In support of this view he describes the behavior of *Ctenolabrus* eggs when they are deprived of oxygen: eggs newly fertilized fail to divide, while those in which segmentation has reached the four-cell stage show a solution of the dividing surface layers, or cell membranes, and a return to a unicellular form. He points out that circumstances which lead to a breakdown of existing membranes would naturally prevent the formation of new ones, and calls attention to the probability that failure to divide depends rather upon the impossibility of membrane formation than upon lack of energy. In favor of this conclusion he mentions the fact that the eggs of a closely related fish, *Fundulus*, the cell membranes of which undergo no solution in an oxygen vacuum, may continue to divide for more than twelve hours.

He observed a similar difference in the two species, in the behavior of the embryonic heart, when its supply of oxygen was gradually diminished. In *Ctenolabrus* the heart was brought to a standstill too suddenly to allow the assumption that lack of energy was the

<sup>1</sup> PFLÜGER: *Archiv f. d. ges. Physiol.*, 1875, x, p. 25.

<sup>2</sup> BUNGE: *Zeitschr. f. physiol. Chemie*, 1874, viii, p. 48.

<sup>3</sup> LOEB: *Archiv f. d. ges. Physiol.*, 1895, lxii, p. 249.

cause, and he supposes that here also there may occur molecular and finally structural changes similar to those seen in the first stages of segmentation, and that these may prevent the conversion of chemical into mechanical energy. The heart of *Fundulus*, on the other hand, beat with increasing slowness until it reached a minimum rate, at which it continued as long as ten hours.

Loeb thinks it possible that the structural changes which occur in the absence of oxygen are the result of the action of abnormal compounds formed under these circumstances, and bases this supposition upon the fact that the products of metabolism are different when no oxygen is present, as had been shown by Araki,<sup>1</sup> who found lactic acid and sugar in the urine of dyspnoeic animals.

Subsequently Broca and Richet,<sup>2</sup> in their study of the anærobic contraction of muscle, arrived at the conclusion that the injurious effects of lack of oxygen which they observed were due to the toxic action of unoxidized waste products.

At Professor Loeb's suggestion, I have subjected *Paramœcia* and other protozoa to the action of various poisons in order to determine whether any of these would produce structural changes similar to those appearing in an oxygen vacuum. With a number of the reagents used such was found to be the case.

***Paramœcium aurelium*.** — The structural changes caused by lack of oxygen have been described by Loeb and Hardesty;<sup>3</sup> but their experiments have been repeated in the present series for the sake of comparison. A few drops of *Paramœcium* culture were enclosed in an Engelmann gas chamber, through which was passed hydrogen that had been washed in potassium hydrate solution, potassium permanganate solution, and water.

After the stream of hydrogen has been passing for several hours, the time varying with the temperature of the room, the animals begin to swim more slowly, they absorb water, the contractile vacuoles increase in size, or several



FIGURE 1.

additional vacuoles may appear, and they sink to the bottom. One or more clear vesicles now protrude from the surface of the animal,

<sup>1</sup> ARAKI: *Zeitschr. f. physiol. Chemie*, 1891, xv, p. 325.

<sup>2</sup> RICHET: *Archives de physiologie*, 1876, viii, p. 829.

<sup>3</sup> LOEB and HARDESTY: *Archiv f. d. ges. Physiol.*, 1895, lxi, p. 583.

and into these some of the vacuoles usually escape; the vesicles finally burst, and the cell contents are extruded (Fig. 1).

If a drop of a 0.1 per cent solution of potassium cyanide be added to several drops of *Paramœcium* culture, there result structural changes which are apparently exactly similar to those described above, but they occur immediately (Fig. 2). If the solution be more concentrated, for instance 0.5 per cent, the



FIGURE 2.

bursting often occurs at once, without a preceding marked change of form.

**Amœba.** — When deprived of oxygen, *Amœba* becomes vacuolated and tends to assume a spherical form (Fig. 3). A dilute solution of antipyrine produces the same effects (Fig. 4). The same is true of digitaline and potassium cyanide solutions.

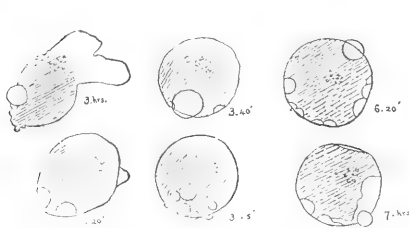


FIGURE 3.

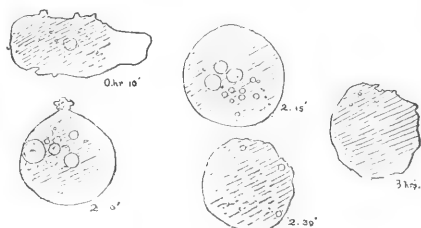


FIGURE 4.

**Oxytricha (?)** — After being subjected for several hours to a stream of hydrogen, this form begins to take up water, and becomes less flattened, the contractile vacuole being much distended. Like *Paramœcia*, it becomes sluggish and gradually sinks, the vacuole contracts, and it remains almost motionless at the bottom. Suddenly



FIGURE 5.

at or near one pole, usually the oral, the surface loses its smooth contour, the surface layer breaks down into small globules, and the change progresses toward the opposite pole, the protoplasm meanwhile becoming detached in spherical masses large and small; these quickly dissolve, and nothing is left of them but scattered granules. As the nuclei pass out they become spherical. The cilia usually, but not invariably, cease moving before the dissolution of the cell wall begins (Fig. 5).

The addition of a dilute solution of potassium cyanide (Fig. 6) or pilocarpine to a culture of oxytricha causes similar changes in structure.

The same changes follow exposure to the action of veratrine (Fig. 7), sulphate of morphia, sulphate of quinine, antipyrine, nicotine, potassium or sodium hydrate, in sufficiently strong solutions, but the cilia usually continue to move until just before that portion of the cell wall upon which they are situated breaks down.



FIGURE 6.

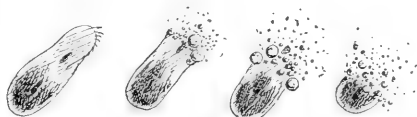


FIGURE 7.

Under the influence of physostigmine (Fig. 8), atropine, or sulphate of strychnia, the aboral pole usually breaks down first, otherwise the visible results are the same.

Jennings<sup>1</sup> has observed that "Paramœcia are not appreciably harmed" by placing them in distilled water. This rather surprising fact must depend upon their offering a great resistance to the entrance of water. The absorption of water, which forms such an important feature in the effects of lack of oxygen, and of poisoning, shows a reduction of this resistance, but probably depends also upon another factor, as is indicated by the following observation (Fig. 9).



FIGURE 8.

Figure 9 represents the changes shown by a sympathetic nerve cell from the Frog, when exposed to a five per cent solution of potassium cyanide. The drawings are from a series of twelve, which were made



FIGURE 9.

with a camera lucida in rapid succession. The osmotic pressure of the potassium cyanide solution being above that of the cell, water

<sup>1</sup> JENNINGS: *Journal of physiology*, 1897, xxi, p. 272.

at first passes out, and the cell shrinks rapidly. The subsequent swelling is probably due to extensive splitting up of molecules within the cell under the influence of the poison, and consequent rise of the intracellular osmotic pressure and absorption of water.

#### CONCLUSIONS.

The visible changes of structure shown by some protozoa when deprived of oxygen may be exactly reproduced by treatment with certain poisons. This indicates that either these poisons prevent oxidation or that lack of oxygen produces toxic substances.

Potassium cyanide, and perhaps other poisons, not only reduce the resistance normally shown by the *Paramœcium* to the entrance of water, but lead to the taking up of water probably by hastening the molecular breakdown and so increasing the osmotic pressure within the cell.

I desire to acknowledge the kind direction of Professor Loeb.

# THE EFFECT OF DISTENTION OF THE VENTRICLE ON THE FLOW OF BLOOD THROUGH THE WALLS OF THE HEART.

By IDA H. HYDE, PH. D.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

IN the course of the experiments "On the relation of the volume of the coronary circulation to the frequency and force of the ventricular contraction in the isolated heart of the cat," made in this Laboratory in 1896,<sup>1</sup> it was incidentally observed that distention of the left side of the isolated heart lessened the volume of the circulation through the coronary vessels, notwithstanding the maintenance of a constant pressure in the aorta. As distention of the heart is a state frequently observed in mountain climbers, athletes, hod-carriers, and many other classes, being indeed almost inseparable from violent, prolonged muscular efforts, and as it constitutes, in its severer forms, a disease of great clinical interest, a systematic pursuit of the clew which Magrath and Kennedy gave is well worth making.

The problem seemed a simple one. The coronary vessels of the isolated heart of the cat should be supplied with defibrinated cat's blood by maintaining a uniform blood-pressure in the aorta, and the outflow from the coronary veins, or, in other words, the volume of the coronary circulation, should be recorded by a suitable apparatus. The effect of the artificial distention of the left side of the heart upon this outflow would then be visible.

The apparatus for accomplishing these ends consisted of a reservoir in which the defibrinated blood could be kept at the temperature of the body; a pressure flask, by which the blood could be driven from the feeding reservoir into the aorta; a mercury manometer, for recording the blood-pressure in the aorta, so that the experimenter could be sure that the driving force remained the same throughout the observation; a membrane manometer, of the Hürthle type, for recording the changes of pressure in the left ventricle; a Mariotte flask, by which the intraventricular pressure could be raised to any height desired; a drop counter, for registering the

<sup>1</sup> MAGRATH, J. B., and H. KENNEDY: *Journal of exper. medicine*, 1897, ii, p. 13.

volume of the coronary circulation; and, finally, a kymograph, with a time recorder.

At the beginning of the experiment, two cats were anæsthetized with ether, tracheotomized, and bled from the left carotid artery. The blood was defibrinated, filtered through glass wool, and placed in the blood reservoirs to warm. Meanwhile, the front wall of the thorax of the second of the two cats was removed, exposing the heart and great vessels; the venæ cavæ and right vena azygos were ligated; cannulas were placed in the aorta, the left subclavian, pul-

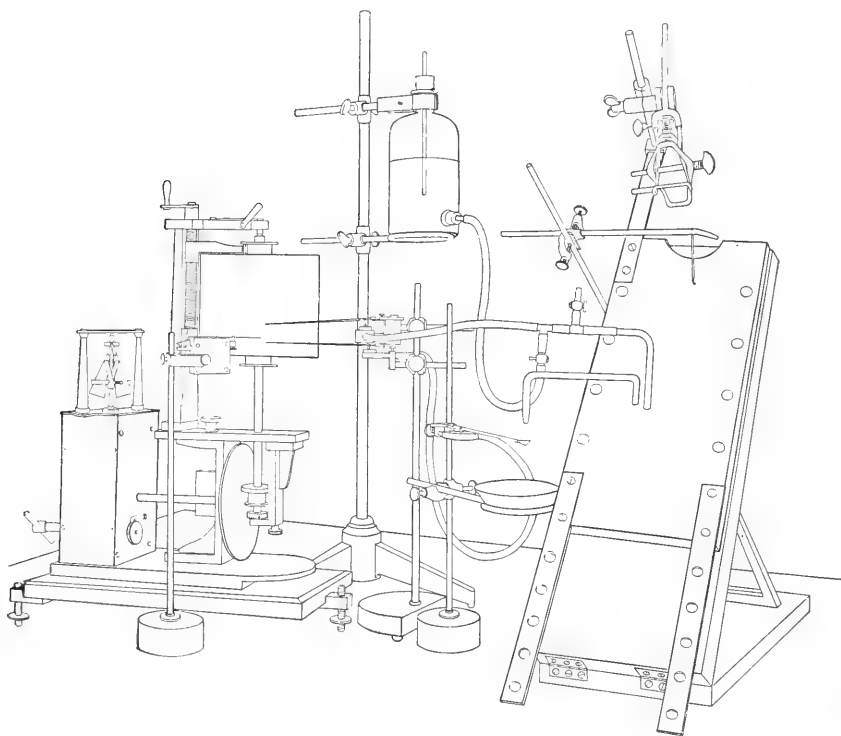


FIGURE 1.

monary, and innominate arteries; and a tube was passed into the left ventricle through the auricular appendix. After washing out the aorta with normal saline solution to remove blood clots and air, the cat, upon its Czermak board, was placed in the inclined stand shown in Fig. 1. At the reader's right, in this figure, is seen the cat-board resting upon an inclined supporting-frame. Two glass tubes are



shown in front and to the left of the cat-board. The lower tube was inserted in the right ventricle through the pulmonary artery, and carried the outflow from the coronary vessels into a porcelain dish. The blood usually escaped from this tube in drops. Each drop, as it fell, struck on a thin aluminium plate of triangular shape, 17 mm. long and 14 mm. wide at the base, fastened on the lever of a Marey tambour. The plate was bent in such a way that the blood could not remain upon it, but ran off into the dish beneath; the impact of the falling drop caused an air wave in the tambour, which was

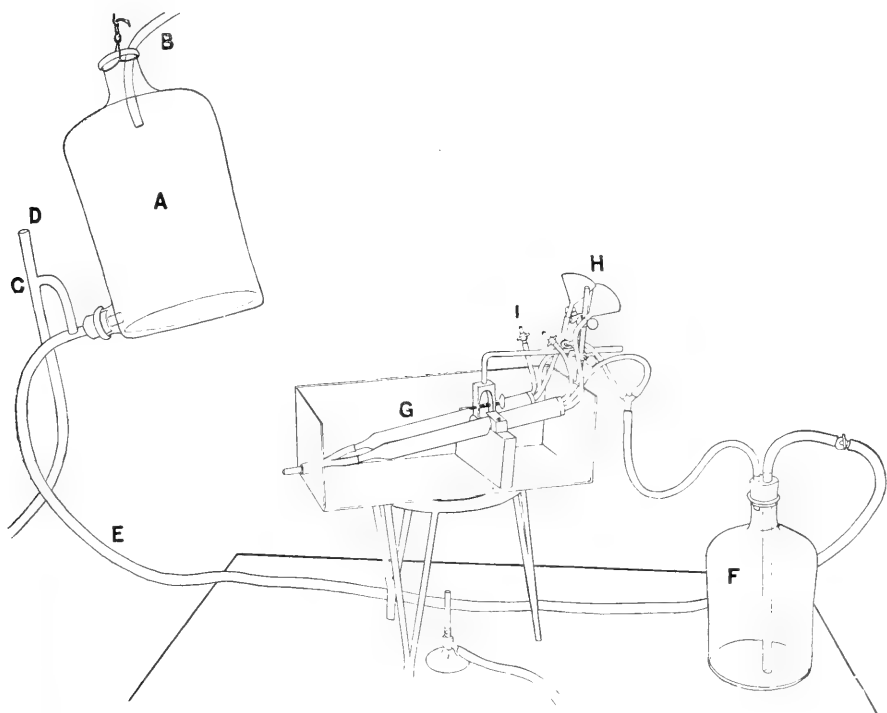


FIGURE 2.

transmitted to a very sensitive recording tambour, the lever of which wrote on the smoked paper of the kymograph. The upper glass tube was inserted in the left ventricle through the left auricular appendix and the mitral valve, and, being filled with normal saline solution, as were the ventricles and the connecting tubes, transmitted the changes of pressure in the ventricle to a Hürthle membrane

manometer, the lever of which wrote on the smoked paper just above the writing of the drop-recorder. A side branch of the manometer led to the atmospheric air and served to place the ventricle at atmospheric pressure. For convenience, this tube has been drawn upright; in actual use, however, the mouth was brought down to the level of the ventricle; the manometer was also at this level. A second side branch led to a Mariotte flask filled with normal saline solution. By raising the flask the pressure in the ventricle could be increased, the amount of pressure being fully recorded by the manometer. The time was recorded by the Jaquet chronometer seen upon the round-base stand in front of the kymograph. The transverse rod, placed at the upper part of the cat-board, supported several hooks which served to lift up the skin at the sides of the thoracic opening, forming thus the walls of a cavity, at the bottom of which lay the heart. This cavity was constantly irrigated with normal saline solution at the body temperature. After filling the cavity, the saline solution escaped into a pan (not shown in the drawing) placed under the supporting-frame.

The apparatus employed for feeding the coronary vessels with blood at the normal temperature and pressure is shown in Fig. 2. The water bottle *A* received a constant stream of tap water through the tube *B*. On reaching the level of the outflow tube *C*, the surplus water passed away into a sink. A constant level was thus maintained in the water bottle. Siphonage was prevented by the upright open tube *D*. A part of the surplus water entered the tube *E*, leading to the pressure bottle *F*, the air in which was subjected to a constant pressure by the column of water in this tube. The compressed air in the pressure bottle was employed to drive the blood from the warming reservoirs through the shortest possible connecting tube into the aorta. The semilunar valves were thereby closed, and, as all the branches of the aorta except the coronary arteries had previously been ligated, the blood was forced through the coronary vessels into the right side of the heart, whence it escaped through a cannula in the pulmonary artery, the venæ cavæ and the vena azygos having been tied. As a rule, the outflow was in drops, and the record of these drops was taken as the measure of the volume of the coronary circulation. Experiments by Magrath and Kennedy,<sup>1</sup> to which the reader is referred, have shown that the size of the drops does not vary enough to make this procedure unsafe.

<sup>1</sup> MAGRATH and KENNEDY: *loc. cit.*, p. 18.

The warming reservoirs, *G*, were filled through the funnel tubes, *H*, the air tubes, *I*, being opened during the filling process. The tubes not furnished with stopcocks were closed with long-handled compression forceps.

The cannula placed in the innominate artery was connected with a mercury manometer, in order that the blood-pressure in the aorta might be observed. The pressure apparatus maintained this pressure with great constancy at any level desired. The manometer served also to assure the observer of the proper closure of the aortic valves. The pressure would fall when these valves were insufficient. At the same time the pressure in the membrane manometer, connected with the interior of the ventricle, would rise. But the aortic valves were seldom insufficient.

The method I have just described is open to the objection that only the blood escaping from the coronary vessels into the right heart was taken as the measure of the volume of the coronary circulation. A slight error is thus introduced, for the coronary blood is discharged not only through the coronary veins but also through the veins of Thebesius, and the veins of Thebesius open into all the chambers of the heart, the left as well as the right. The method employed by me registered the outflow from the right heart, but omitted that from the left. Furthermore, the normal saline solution used to distend the left side of the heart could enter the veins of Thebesius present on this side, pass through the branches that connect them with the coronary veins, and flow with the coronary blood into the right heart, thus making part of the recorded outflow. Yet these errors did not seriously impair the method for the purpose for which it was devised, as the number of the vessels of Thebesius opening into the left heart is too small, and the circulation through them too slight, to be of practical importance in these experiments.<sup>1</sup>

A factor of much greater difficulty was the unexpectedly powerful stimulation of the ventricular muscle by the distention of the ventricle. Hearts that had ceased to contract, although fed through their coronary vessels with defibrinated blood at uniform temperature and pressure, often suddenly awoke to new contractions when the intra-ventricular pressure was raised; and hearts that were beating feebly

<sup>1</sup> The circulation through the vessels of Thebesius from the left ventricle and auricle, though relatively too small to affect seriously the method here described, may be of importance in the nutrition of hearts in which the coronary arteries are obstructed. See the experiments of F. H. Pratt, this Journal, vol. i, p. 86.

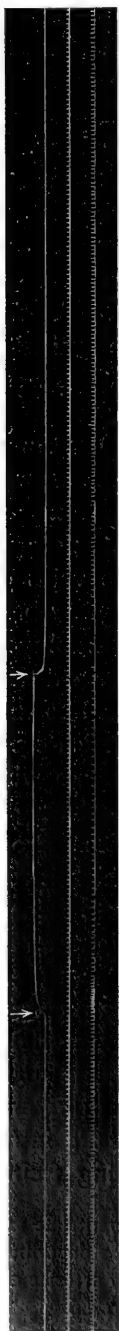


FIG. 3. One half the original size. The flow through the coronary vessels lessens when the left ventricle is distended. The uppermost curve is the pressure in the left ventricle; the middle curve gives the time in seconds; the lowest curve is the volume of the coronary circulation in drops. The first arrow points to the raising of the pressure in the left ventricle; the second, to the lowering of the pressure to that of the atmosphere again.

before distention were often excited to powerful efforts by the stimulus of distention. The increase in the force of beat in consequence of distention seemed at first of no importance, — nothing being then definitely known of the effect of ventricular contraction on the flow of blood through the heart walls. But when, a short time after my experiments were begun, Dr. W. T. Porter<sup>1</sup> proved that the contraction of the ventricle compresses the vessels in its walls, drives out their contents, and forces along the coronary blood, like the strokes of a pump, it was seen that my method could give a pure result only when used with non-beating hearts or with contracting hearts in which distention either failed, for some reason, to stimulate the ventricle to increased action, or overcame the effect of the increased contraction and lessened the volume of the coronary circulation in spite of the favorable influence of the greater force of beat.

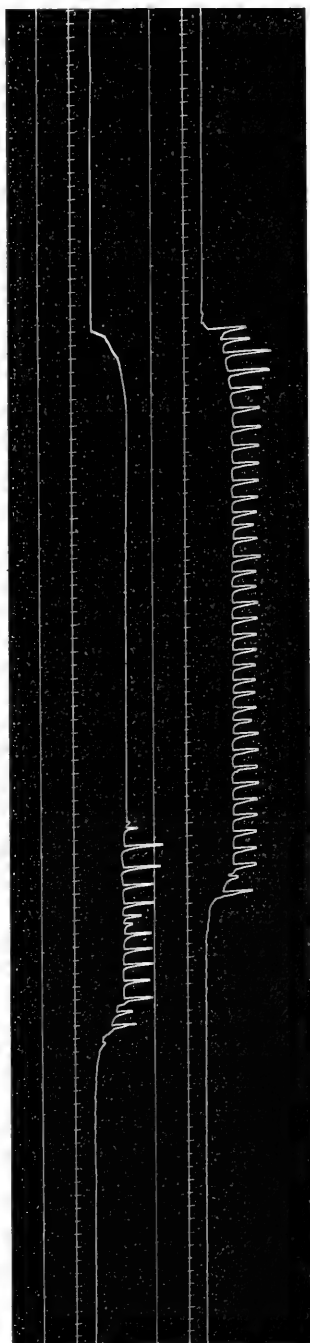
The discovery of the force-pump action of the ventricle bore against the method in yet another way. Magrath and Kennedy had shown that lessening the volume of the coronary circulation in the isolated heart lessened the force of contraction, and, to a slight extent, the frequency as well. Hence the distention of the heart, by diminishing the volume of the coronary circulation, may, if the heart is not stimulated to increased contraction by the stimulus of distention, cut down the force and frequency of contraction, and thus, secondarily, still further reduce the coronary flow. But this error is not sufficiently large to be of moment in the present investigation.

The foregoing considerations divide the ques-

<sup>1</sup> PORTER, W. T.: The influence of the heart-beat on the flow of blood through the walls of the heart. This Journal, i, p. 145.

tion in hand into two parts. It is necessary to determine first, whether the distention of the heart checks the flow of blood through the coronary vessels when the organ is at rest. The answer to this question is furnished by Fig. 3, from the experiment of October 24, 1896. The uppermost curve in this figure was drawn by a Hürthle membrane manometer connected with the left ventricle. The rise of three millimetres in this curve indicates an increase of 15 mm. Hg intraventricular pressure; this distention of the ventricle was accomplished by opening a side branch of the ventricular cannula tube, in the manner described on page 218. The middle curve gives the time in seconds. The lowest curve records the number of drops of blood escaping from the coronary vessels into the right heart, — practically, the volume of the coronary circulation. The pressure in the aorta was 78 mm. Hg. When the pressure in the left ventricle is increased, a part of the contents of the coronary vessels is squeezed out, so that the first five or six seconds of the period of distention are marked by an increase in the flow. When the pressure in the ventricle is lowered again, at the second arrow, the sudden dilation of the vessels checks the flow during a few seconds. Apart from these temporary effects, the consequence of

FIG. 4. Two thirds the original size. Two records of the effect of distention of the left ventricle on the flow through the coronary vessels. In each record, the uppermost curve is the pressure in the left ventricle; the middle curve, the time in seconds; and the lowest curve, the number of drops of blood flowing through the heart walls.



distention is a considerable diminution in the volume of the coronary flow.

The number of drops per 20 seconds throughout the experiment was as follows, beginning 60 seconds before distention:—

1-20 seconds,	23 drops.
21-40       “	23   “
41-60       “	23   “

Distention of the left ventricle at 60th sec.

61- 80 seconds,	26 drops.
81-100       “	15   “
101-120      “	14   “
121-140      “	8     “

At the 126th second, atmospheric pressure was restored.

141-160 seconds,	20 drops.
161-180       “	24   “
181-200       “	24   “
201-220       “	23   “
221-240       “	23   “
241-260       “	22   “

The distention of the non-beating heart, therefore, diminishes the flow of blood through the coronary vessels.

I pass now to the effect of distention of the non-beating heart spurred into activity by the stimulus of distention. Fig. 4 illustrates one of these experiments. Two records of the effect of distention on the flow through the coronary vessels are here given. The uppermost curve in each set of three is the pressure in the left ventricle, recorded as described above; the middle curve gives the time in seconds, and the lowest curve the number of drops of blood escaping from the coronary vessels. The stimulating effect of the distention of the heart is very well shown. In the upper intraventricular curve, the pressure rises 27 mm. Hg; in the lower, 35 mm. Hg. In the first experiment (lower tracing), the distention at first fails to call forth contractions; in the second (upper tracing), the heart begins to beat as soon as distended. In spite of the favorable action of the contractions upon the coronary circulation, the effect of the distention, aside from the momentary squeezing out of a part of the contents of the vessels when the pressure in the ventricle is first raised, is to diminish the flow through the coronary vessels.

The second of the two problems before us, namely, whether distention checks the coronary flow in beating hearts, in spite of the favorable influence exerted on the coronary flow by the increased force of

beat called forth by the distention, is already partly answered by the preceding experiment. A more satisfactory reply is afforded by the experiment recorded in Fig. 5. This heart was beating very well before the ventricle was distended. The distention was purposely made very slight, and, as will be seen on examining the curve, is certainly not greater than that observed in hearts distended by natural causes in the intact animal. The base line of the intraventricular pressure-curve rises not more than 5 mm. Hg. The distention increased the force of beat to some extent, the upstrokes becoming taller, but the flow through the coronary arteries fell off nevertheless. The number of drops per twenty seconds was as follows:

1-20 seconds,	22 drops.
21-40        "	22       "

The ventricle was distended at the 40th second of this record.

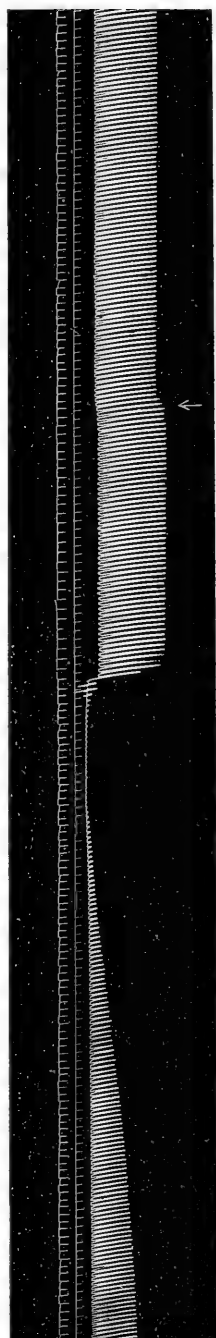
41-60 seconds,	18 drops.
61-80        "	19       "

The pressure in the ventricle was lowered at the 73d second. From the 61st to the 73d second the rate of flow was 18 drops per 20 seconds.

81-100 seconds,	24 drops.
101-120       "	23       "
121-140       "	23       "

The aortic pressure during the observation was 64 mm. Hg. This curve illustrates also the curious weakening—in some cases amounting to absolute disappearance of the heart-beat—immediately after the distention is withdrawn. The ventricle seems to recover somewhat slowly from this exhaustion.

FIG. 5. Two thirds the original size. The uppermost curve is the pressure in the left ventricle; the middle curve, the time in seconds; the lowest curve, the volume of the coronary circulation. The arrow marks the raising of the intraventricular pressure.



It appears, then, that moderate distention may diminish the flow of blood through the walls of even the contracting heart.

These experiments have demonstrated, therefore, that the volume of the coronary circulation is diminished by the distention of the ventricle. This diminution, however, is overcome, in some cases, by the increase consequent on the greater force or frequency with which the ventricle may contract in response to the stimulus of distention.

My material is not sufficient for a discussion of the manner in which the diminished coronary flow is brought about, whether by mechanical influences or by the action of vasomotor mechanisms.

In conclusion, it is with great pleasure that I acknowledge my indebtedness to Dr. H. P. Bowditch, for permission to work in the Laboratory of the Medical School, and to Dr. W. T. Porter, at whose suggestion the investigation was undertaken, and who was constantly ready with kind advice and assistance.



## THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF SOME EDIBLE AMERICAN FUNGI.

By LAFAYETTE B. MENDEL.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

THE collection and spreading of information regarding fungi has lately received considerable attention. The efforts in this direction have been confined for the most part to descriptions of the common species, their peculiarities of growth and distribution. Especial consideration has been devoted to the variations among the fungi as regards toxicity; but accurate statements regarding chemical composition and possible nutritive value are largely wanting. The following extract from a recent monograph will serve to illustrate the current opinions. In referring to the edible mushrooms, it states: "The general opinion is, that mushrooms constitute a very nutritious and sustaining diet. Chemical analysis and personal experience indicate this. The former has shown that in their dry matter they contain from twenty to fifty per cent of protein or nitrogenous material. They may, therefore, well be called a kind of vegetable meat, and be used as a substitute for animal food. Like other vegetables they are largely composed of water, which is from eighty to ninety per cent of the whole. . . . The presence of so much nitrogenous material induces rapid decay and loathsome decomposition in them. . . . A hearty meal on mushrooms alone would be about as reasonable as a dinner on nothing but beefsteak, and might be expected to be followed by similar ill consequences."<sup>1</sup>

In view of the increasing importance of fungi as articles of diet, the writer has gladly followed the suggestion of Professor Chittenden to ascertain something more definite regarding the composition of edible mushrooms, with particular reference to their nutritive qualities.

<sup>1</sup> PECK, C. H.: Report of the New York State Botanist, 1895, p. 113. Cf. also PECK: Mushrooms and their uses, 1897, p. 4. For the source of the statements quoted Professor Peck has referred the writer to the Atlas of Champignons, by Richon and Rosé, and to Les Champignons, by Cordier — both of which it has been impossible to consult.

**Methods.**—Specimens were obtained from various sources, and in some instances different samples of the same species were examined.<sup>1</sup> The common methods of studying the composition of agricultural products have been adopted, the directions given by the Association of Official Agricultural Chemists being closely followed in most instances.<sup>2</sup> The mushrooms were cut up finely and thoroughly mixed. Samples were taken for the determination of moisture, while the bulk of the material was dried on a water bath and then ground up to a fine powder. Dried to constant weight at 105° C., this served as material for analysis.

*Ash* was determined in the usual way, the incineration being carried on with the lowest possible heat. The mushrooms employed were previously cleaned with considerable care, and thus an excess of inorganic impurity, such as sand, was avoided.

*Ether extract* was obtained by treating the material with anhydrous and alcohol-free ether in a Soxhlet extractor for sixteen hours, the extract being finally dried in vacuo to constant weight. Recently Bugdanow<sup>3</sup> has shown that this method is insufficient to remove the last traces of fats completely from some vegetable materials, even when they are finely divided. The error is not sufficiently large, however, to affect the general conclusions from the analyses. In order to examine the extract for cholesterin it was saponified in the usual way with alcoholic potash. Cholesterin (or closely allied substances) was detected by Salkowski's reaction; but the method of separation employed obviously does not exclude the possibility of this substance existing in combination with fatty acids in the fungi.<sup>4</sup>

*Crude fibre* was determined according to Wiley's method,<sup>5</sup> in the residue left after extractions with ether.

*Total nitrogen* was found by the Kjeldahl method, duplicate determinations always showing a very close agreement. It is customary in agricultural analysis to express the results thus obtained, and

<sup>1</sup> Acknowledgment is gratefully made of specimens obtained through the courtesy of Mr. Hollis Webster, of Cambridge, and Captain McIlvaine, of Philadelphia. The material used has in every case been identified, or verified, by Dr. A. W. Evans, to whom our thanks are due.

<sup>2</sup> See WILEY, H. W.: *Agricultural analysis*, 1897.

<sup>3</sup> BUGDANOW: *Archiv f. d. ges. Physiol.*, 1897, lxxviii, p. 408.

<sup>4</sup> Cf. HÜRTHE, K.: *Ueber die Fettsäure-Cholesterin-Ester des Blutes*. *Zeitschr. für physiol. Chemie*, 1896, xxi, p. 352.

<sup>5</sup> WILEY: *Agricultural analysis*, 1897, iii, p. 304.

multiplied by the factor 6.25, as "crude protein." The latter term is thus made to include albuminoids and extractive bodies as well as the proteids proper.<sup>1</sup> Not only do these individual groups possess quite variable significance as foods, but this investigation has further demonstrated that such calculations may lead to quite erroneous conclusions. In the mushrooms, at least, a considerable part of the nitrogen probably exists as non-proteid nitrogen, a portion even belonging to the so-called crude-fibre, or cellulose elements of the fungi.<sup>2</sup> In a large number of our analyses an attempt has been made to separate the nitrogen of the extractive bodies (amide-nitrogen, etc.) by treating a portion of the material repeatedly with boiling 85 per cent alcohol, so long as anything could be removed. The nitrogen content of the alcoholic extract having been determined, and then calculated on the material used, is designated as *extractive nitrogen*.<sup>3</sup> The amount of *alcohol soluble material* was ascertained at the same time, by filtering the undissolved extraction residues on weighed filters and drying at 105° C. to constant weight. The difference between the total nitrogen and extractive nitrogen is provisionally given as *protein nitrogen*, though, as stated above, there is at present no justification for expressing the results as pure protein. Indeed, as will be pointed out later, this so-called protein nitrogen, in the present instance, contains a large proportion of nitrogen in a form wholly unavailable for the nutrition of the body.

*Soluble carbohydrates* were determined in an approximate manner by extracting the dry substance repeatedly with hot water and then boiling the extract for ten hours with hydrochloric acid of two per cent resulting strength. The sugar was determined as dextrose in the neutralized fluid, by the Allihn gravimetric method.

**Experimental Data.** — *Coprinus comatus* (Shaggy coprinus). The specimens were freshly gathered and had not yet turned "inky." They varied very widely in size, thirty-six mushrooms weighing 1485 grams, of which 980 grams belonged to the caps (pileus) and 505 grams to the stems.

<sup>1</sup> Cf. ATWATER, W. O.: Foods. Nutritive value and cost. Farmers' bulletin, No. 23, pp. 5. 6. U. S. Dept. of Agriculture, 1894.

<sup>2</sup> WINTERSTEIN: Berichte der deutsch. botan. Gesellsch., xi, p. 441; also Zeitschr. für physiol. Chemie, 1894, xix, p. 521; 1895, xxi, p. 134; GILSON: La cellule, xi, 1er fascicule.

<sup>3</sup> Cf. MÖRNER, C. Th.: Zeitschr. für physiol. Chemie, 1886, x, 506.

The average weight of a fresh specimen was thus :

Pileus . . . . .	27 grams.
Stem . . . . .	14 "
Total weight . . . . .	41 "

A specimen which had attained the average growth weighed :

Pileus . . . . .	43 grams.
Stem . . . . .	25 "
Total weight . . . . .	68 "

An analysis yielded the following results :

Water . . . . .	92.19 per cent.
Total solids . . . . .	7.81 "

The dry substance contained :

Total nitrogen . . . . .	5.79 per cent.
Extractive nitrogen . . . . .	3.87 "
Protein nitrogen . . . . .	1.92 "
Ether extract . . . . .	3.3 "
Crude fibre . . . . .	7.3 "
Ash . . . . .	12.5 "
Material soluble in 85 per cent alcohol . . . . .	56.3 "

*Coprinus atramentarius* (Inky coprinus). Two separate, freshly gathered lots of this species were examined. The one (a) contained six young small specimens weighing 5.5 grams, or 0.9 gram each; the other (b) contained eight mushrooms weighing 12 grams, or 1.5 grams each. An analysis gave :

	a. Per cent.	b. Per cent.
Water . . . . .	92.31	94.42
Total solids . . . . .	7.69	5.58

The dry substance contained :

Total nitrogen . . . . .	4.68	4.77
Ether extract . . . . .	3.1	5.7
Crude fibre . . . . .	9.3	....
Ash . . . . .	16.8	20.1

*Morchella esculenta* (Common morel). Two lots of this species were obtained from Stockbridge,

Mass. (a) The specimens were of full size. Thirteen morels weighed 195 grams, or an average of 15 grams each. (b) Small, young morels. An analysis gave :

	a. Per cent.	b. Per cent.
Water . . . . .	89.54	91.24
Total solids . . . . .	10.46	8.76

The dry substance contained :

Total nitrogen . . . . .	4.66	5.36
Extractive nitrogen . . . . .	1.17	....
Protein nitrogen . . . . .	3.49	....
Ether extract . . . . .	4.8	7.5
Crude fibre . . . . .	8.7	9.5
Ash . . . . .	10.4	13.6
Material soluble in 85 per cent alcohol . . . . .	29.3	....

In the same species Pizzi<sup>1</sup> has found 0.575 per cent nitrogen, a figure in close agreement with the above results when calculated upon the fresh material, viz. (a) 0.48 per cent N; (b) 0.47 per cent N.

*Polyporus sulphureus* (Sulphury polyporus). The specimens were obtained from Pennsylvania. An analysis gave :

Water . . . . .	70.80 per cent.
Total solids . . . . .	29.20 "

The dry substance contained :

Total nitrogen . . . . .	3.29 per cent.
Extractive nitrogen . . . . .	1.06 "
Protein nitrogen . . . . .	2.23 "
Ether extract . . . . .	3.2 "
Crude fibre . . . . .	3.0 "
Ash . . . . .	7.3 "
Material soluble in 85 per cent alcohol . . . . .	27.8 ....

*Pleurotus ostreatus* (Oyster mushroom). This mushroom is obtainable in large quantities, and though somewhat tough in texture, is universally classed with the edible species.

<sup>1</sup> PIZZI: Botanischer Jahresbericht, 1889, p. 316.

Specimens gathered from a tree in New Haven contained:

Water . . . . .	73.70	per cent.
Total solids . . . . .	26.30	"

The dry substance contained:

Total nitrogen . . . . .	2.40	"
Extractive nitrogen . . . . .	1.27	"
Protein nitrogen . . . . .	1.13	"
Ether extract . . . . .	1.6	"
Crude fibre . . . . .	7.5	"
Ash . . . . .	6.1	"
Material soluble in 85 per cent alcohol . . . . .	31.5	"

*Clitocybe multiceps*. Peck. The material was collected near Boston, in June, 1897. A portion of small, young specimens was analyzed separately. The results follow:

	Young Specimens. Per cent.	Full-grown Specimens. Per cent.
Water . . . . .	89.61	93.49
Total solids . . . . .	10.39	6.51
The dry substance of the full-grown specimens contained:		
Total nitrogen . . . . .	5.36	per cent.
Extractive nitrogen . . . . .	3.38	"
Protein nitrogen . . . . .	1.98	"
Ether extract . . . . .	6.0	"
Crude fibre . . . . .	9.6	"
Ash . . . . .	11.5	"
Material soluble in 85 per cent alcohol . . . . .	57.2	"

A portion of the mushrooms was separated into stems and caps and each analyzed separately, with the following results:

	Stem. Per cent.	Pileus. Per cent.
Water . . . . .	94.07	92.68
Total solids . . . . .	5.93	7.32
Total nitrogen in dry substance . . . . .	3.92	5.84
Ash in dry substance . . . . .	12.98	10.82

The relatively higher content of nitrogen in the pileus corresponds with the distribution of proteid as shown by histochemical examination. In *Agaricus campestris*, *Boletus edulis*, and *Boletus scaber*, C. Th. Mörner has found similar differences between the nitrogen content of caps and stems.<sup>1</sup>

*Hypholoma candolleianum*.<sup>2</sup> The specimens were obtained from East Milton, Mass., in June, 1897. A few small, young specimens were also obtained from Brookline, Mass. Analyses follow:

	Full-grown Specimens. Per cent.	Younger Specimens. Per cent.
Water . . . . .	88.97	91.97
Total solids . . . . .	11.03	8.03
The dry substance contained:		
Total nitrogen . . . . .	4.28	4.44
Extractive nitrogen . . . . .	1.79	....
Protein nitrogen . . . . .	2.49	....
Ether extract . . . . .	2.5	....
Crude fibre . . . . .	12.1	....
Ash . . . . .	13.9	19.9
Material soluble in 85 per cent alcohol . . . . .	44.4	....

*Agaricus campestris* (Common mushroom). Two varieties of the common mushroom were collected in New Haven. Fifteen specimens of one variety weighed 42 grams, an average weight of 2.8 grams each. The analysis gave:

	a. Per cent.	b. Per cent.
Water . . . . .	87.88	92.20
Total Solids . . . . .	12.12	7.80
Total nitrogen in dry substance . . . . .	4.42	4.92
Ash in dry substance . . . . .	11.66	17.18

<sup>1</sup> MÖRNER, C. Th.: Zeitschr. für physiol. Chemie, 1886, x, p. 510.

<sup>2</sup> The specimens corresponded with those described under this name by Stevenson in his work on British Hymenomycetes. Mr. Hollis Webster has informed the writer that Professor Farlow is inclined to regard them as *H. appendiculatum*.

Regarding the differences in nitrogen content of cap and stem, compare the remarks under *Clitocybe multiceps*.

*Marasmius oreades* (Fairy-ring mushroom). Twenty freshly gathered specimens (from New Haven) weighed 9 grams, an average weight of 0.45 grams each. The analysis gave:

Water . . . . .	74.96 per cent.
Total solids . . . . .	25.04 "

Total nitrogen of dry substance . . . . .	5.97	"
Ash of dry substance . . . . .	7.23	"

*Cortinarius collinitus* (Smeared cortinarius). Young specimens gathered in New Haven early in November, 1897. The analysis gave:

Water . . . . .	91.13 per cent.
Total solids . . . . .	8.87 "

Total nitrogen of dry substance . . . . .	3.63	"
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**Digestion Experiments.**—In order to procure further data regarding the nutrient value of the mushrooms, artificial digestion experiments were carried out with seven species of the fungi. The procedure was modified after the Stutzer method. About 2.5 grams dry substance were treated in a flask with 100 c.c. of an artificial gastric juice, containing 0.1 gram very active scale pepsin and having an acidity of 0.35 per cent HCl. The flasks were frequently shaken, and after remaining in a thermostat at 38° C. for twelve hours, the undissolved residue was filtered off, washed free from acid, and again treated in the flask for several hours at 38° C. with 100 c.c. amylolytically active fresh chloroform-water extract of dog's pancreas, a little chloroform being added to prevent putrefaction or fermentation. Sodium carbonate (0.25 gram) was then added, followed by 25 c.c. of a proteolytically active thymolized extract of dry pancreas powder (Kühne's method.<sup>1</sup>) At the end of seven hours the residue was again filtered on a weighed filter, washed thoroughly with hot water, and dried at 105° C. to constant weight. *Undigested residue* was thus determined, and the nitrogen content ascertained by the Kjeldahl method and expressed as *nitrogen in residue*. The results expressed in percentages of dry substance are tabulated below.

**Discussion of the Analytical Data.** *Nitrogen and Protein.* From the results obtained it is evident that the nitrogen (and proteid) content of the mushrooms (or at least those species examined) is considerably smaller than is ordinarily stated. Thus Pavy, quoting from Payen's analyses, announces that in the dried state "mushrooms contain 52 per cent, morels 44 per cent, white truffles 36 per cent, black truffles

<sup>1</sup> See CHITTENDEN and CUMMINS: Studies from the laboratory of physiological chemistry, Yale University, i, p. 109.

SPECIES DIGESTED.	Dissolved substance.	Undigested residue.	Nitrogen in residue.	Total nitro- gen.	Total nitrogen soluble.	Total nitrogen insoluble.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
<i>Coprinus comatus</i> . . .	73.79	26.21	4.21	5.79	4.69	1.10
“ <i>atramentarius</i> . . .	71.84	28.16	2.79	4.68	3.90	0.78
<i>Clitocybe multiceps</i> . . .	62.43	37.57	1.96	5.36	4.63	0.73
<i>Hypholoma candolleianum</i>	68.02	31.98	3.63	4.28	3.12	1.16
<i>Morchella esculenta</i> . . .	50.58	49.42	4.16	4.66	2.61	2.05
<i>Pleurotus ostreatus</i> . . .	40.57	59.43	1.39	2.40	1.58	0.82
<i>Polyporus sulphureus</i> . . .	45.00	55.00	1.05	3.29	2.71	0.58

31 per cent, nitrogenous matter.”<sup>1</sup> In a number of species we have determined not only the total nitrogen, but also the extractive (non-proteid) nitrogen as well as the nitrogen in the residue insoluble after artificial gastric and pancreatic digestion. The “protein” nitrogen multiplied — after deduction of the nitrogen in the undigested residue — by the factor 6.25 will give an approximation to the amount of proteid material available through the digestive processes going on in the alimentary canal, and thus throw some light on the true nutritive value of the mushrooms. It is here assumed that the nitrogenous bodies soluble in alcohol are likewise soluble in the digestive fluids; as to the possible presence of alcohol soluble proteids like zein, gliadin, etc., definite information is wanting at present.

The first table following gives a summary of the nitrogen content of various species; in the second table the amount of available proteid has been calculated in the manner referred to.

In considering the relatively high nitrogen content of the residue resisting digestion, it is to be noted that this is not necessarily derived from unattacked proteids. Winterstein<sup>2</sup> and others have shown that the “cellulose” preparations obtained by the usual methods from various fungi contain a considerable percentage of nitrogen; thus a preparation from *Boletus edulis* contained 5.5 per cent N, and this substance, like similar preparations from *Agaricus campestris*, *Morchella esculenta* and other forms, yields glycosamin,  $C_6H_{11}O_5.NH_2$ , on de-

<sup>1</sup> PAVY: Food and dietetics, 1881, p. 187.

<sup>2</sup> WINTERSTEIN: *loc. cit.*; also, Berichte der deutschen chemischen Gesellschaft, 1894, xxvii, p. 3113; xxviii, p. 167.

composition with HCl. It is thus allied to the chitin found in the animal kingdom; further investigation in this direction is highly desirable.

## I.

The percentages are calculated on the dry substance.	Total nitrogen.	Extractive nitrogen.	"Crude protein" nitrogen.
	Per cent.	Per cent.	Per cent.
<i>Coprinus comatus</i> . . . . .	5.79	3.87	1.92
<i>Pleurotus ostreatus</i> . . . . .	2.40	1.27	1.13
<i>Morchella esculenta</i> . . . . .	4.66	1.17	3.49
<i>Hypholoma candolleianum</i> . . . . .	4.28	1.79	2.49
<i>Clitocybe multiceps</i> . . . . .	5.36	3.38	1.98
<i>Polyporus sulphureus</i> . . . . .	3.29	1.06	2.23
<i>Agaricus campestris</i> — <i>a</i> . . . . .	4.42	....	....
"      " <i>b</i> . . . . .	4.92	....	....
<i>Coprinus atramentarius</i> — <i>a</i> . . . . .	4.68	....	....
"      " <i>b</i> . . . . .	4.77	....	....
<i>Morchella esculenta</i> (young) . . . . .	5.36	....	....
<i>Marasmius oreades</i> . . . . .	5.97	....	....
<i>Cortinarius collinitus</i> . . . . .	3.63	....	....
<i>Hypholoma candolleianum</i> (young) . . . . .	4.44	....	....

## II.

The percentages are calculated on the dry substance.	Nitrogen insoluble in 85% alcohol.	Nitrogen in residue from digestion.	Nitrogen of proteid dissolved in digestion.	Digestible proteid (N X 6.25).
	Per cent.	Per cent.	Per cent.	
<i>Morchella esculenta</i> . . . . .	3.49	2.05	1.44	<b>9.00</b>
<i>Hypholoma candolleianum</i> . . . . .	2.49	1.16	1.33	<b>8.31</b>
<i>Coprinus comatus</i> . . . . .	1.92	1.10	0.82	<b>5.12</b>
<i>Clitocybe multiceps</i> . . . . .	1.98	0.73	1.25	<b>7.81</b>
<i>Polyporus sulphureus</i> . . . . .	2.23	0.58	1.65	<b>10.31</b>
<i>Pleurotus ostreatus</i> . . . . .	1.13	0.82	0.31	<b>1.94</b>



It is of interest in this connection to compare the results obtained by C. Th. Mörner<sup>1</sup> in an investigation of thirteen species of fungi common in Sweden. Nitrogenous constituents alone were considered, total N and extractive N, as well as digestible and indigestible N being determined by methods analogous to those used in the present research. Mörner's results, summarized in the following table, show a close agreement, in general, with those already given for different American species.

The results are expressed as percentage of dry substance.	N soluble in pancreatic juice.	N soluble in gastric juice.	Digestible Protein-N.	Indigestible Protein-N.	Protein-N.	Extractive-N.	Total N.
<i>Agaricus procerus</i> (cap) . . . .	0.28	2.71	2.99	1.27	4.21	2.02	6.23
<i>Agaricus campestris</i> (cap) . . .	0.35	3.29	3.64	1.17	4.89	2.49	7.38
“ “ (stem) . . . .	0.10	2.78	2.88	1.09	4.04	1.98	6.02
<i>Lactarius deliciosus</i> . . . . .	0.21	1.20	1.41	1.05	2.51	0.60	3.11
“ <i>torminosus</i> . . . . .	0.17	0.79	0.96	1.00	1.94	0.58	2.52
<i>Cantharellus cibarius</i> . . . . .	0.08	0.71	0.79	1.46	2.29	0.40	2.69
<i>Boletus edulis</i> (cap) . . . . .	0.16	1.94	2.10	0.65	2.73	1.14	3.87
“ “ (stem) . . . . .	0.14	1.62	1.76	0.67	2.35	0.95	3.30
“ <i>scaber</i> (cap) . . . . .	0.18	1.48	1.66	0.85	2.54	0.58	3.12
“ “ (stem) . . . . .	0.12	0.87	0.99	0.62	1.71	0.48	2.19
“ <i>luteus</i> . . . . .	0.22	0.48	0.70	1.06	1.77	0.74	2.51
<i>Polyporus ovinus</i> . . . . .	0.08	0.42	0.50	0.84	1.35	0.45	1.80
<i>Hydnum imbricatum</i> . . . . .	0.08	0.77	0.85	0.76	1.59	0.96	2.55
“ <i>repandum</i> . . . . .	0.15	1.08	1.23	1.55	2.78	0.74	3.52
<i>Sparassis crispa</i> . . . . .	0.09	0.37	0.46	0.40	0.97	0.21	1.18
<i>Morchella esculenta</i> . . . . .	0.22	1.97	2.19	1.90	4.18	0.81	4.99
<i>Lycoperdon Bovista</i> . . . . .	....	3.13	3.13	2.70	5.79	2.40	8.19

**Ether Extract.**—The amount of ether extract varied from 1.6 to 7.5 per cent in different species, as shown in the following summary of results.

<sup>1</sup> MÖRNER, C. Th.: *Zeitschr. für physiol. Chemie*, 1886, x, p. 503.

## ETHER EXTRACT.

SPECIES.	Percentage calculated on dry substance.	Cholesterin.
<i>Morchella esculenta</i> (young) . . . . .	7.5	Present.
<i>Clitocybe multiceps</i> . . . . .	6.0	"
<i>Morchella esculenta</i> . . . . .	4.8	"
<i>Coprinus comatus</i> . . . . .	3.3	"
<i>Polyporus sulphureus</i> . . . . .	3.2	"
<i>Coprinus atramentarius</i> . . . . .	3.1	"
<i>Hypholoma candolleum</i> . . . . .	2.5	"
<i>Pleurotus ostreatus</i> . . . . .	1.6	"

Gérard<sup>1</sup> examined the extract from *Lactarius vellereus* and *L. piperratus*, and found oleic and stearic acids present both as glycerides and as free acids. Volatile fatty acids were also obtained, together with cholesterol or a closely related body (ergosterin), and evidences of lecithin. In the present research both fats and free fatty acids were found, and cholesterol reactions were obtained in every instance, the quantitative relations apparently varying considerably in the different species.

**Alcohol Extract.**—The following summary shows the amount of material soluble in warm 85 per cent alcohol in a number of species.

## ALCOHOL EXTRACT.

The percentages are calculated on the dry substance.	Percentage of soluble material.	Percentage of nitrogen dissolved.
<i>Clitocybe multiceps</i> . . . . .	57.2	3.38
<i>Coprinus comatus</i> . . . . .	56.3	3.87
<i>Hypholoma candolleum</i> . . . . .	44.4	1.79
<i>Pleurotus ostreatus</i> . . . . .	31.5	1.27
<i>Morchella esculenta</i> . . . . .	29.3	1.17
<i>Polyporus sulphureus</i> . . . . .	27.8	1.06

<sup>1</sup> GÉRARD: *Journal de pharmacie et de chimie*, 1890, 5 Série, xxi, p. 408; *ibid.* 1891, xxiii, p. 7. References to the earlier literature will be found in the first of these papers.

**Inorganic constituents.** — The amount of ash varied somewhat, as shown in the table below. Among the bases present, K, Na, and sometimes Ca are to be found, the K being quite abundant. Iron was always present. Of acids, phosphoric and sulphuric predominated, chlorine being occasionally found.

ASH.

The percentages are calculated on the dry substance.	Per cent.
<i>Coprinus atramentarius</i> — <i>a</i> . . . . .	16.8
“ “ — <i>b</i> (young) . . . . .	20.1
“ <i>comatus</i> . . . . .	12.5
<i>Hypholoma candolleianum</i> — <i>a</i> . . . . .	13.9
“ “ — <i>b</i> (young) . . . . .	19.9
<i>Morchella esculenta</i> — <i>a</i> . . . . .	10.4
“ “ — <i>b</i> (young) . . . . .	13.6
<i>Agaricus campestris</i> — <i>a</i> . . . . .	11.7
“ “ — <i>b</i> . . . . .	17.2
<i>Clitocybe multiceps</i> . . . . .	11.5
“ “ (stems) . . . . .	13.0
“ “ (pileus) . . . . .	10.8
<i>Polyporus sulphureus</i> . . . . .	7.3
<i>Marasmius oreades</i> . . . . .	7.2
<i>Pleurotus ostreatus</i> . . . . .	6.1

**Crude Fibre.** — Under this name is included the residue resistant to boiling acids and alkalis, and scarcely to be considered as homogeneous in nature. The results of the analyses are tabulated below.

It has already been pointed out that the cellulose of the fungi contains nitrogen in many instances, and Winterstein<sup>1</sup> has shown that the latter is not due to proteids or nucleins mechanically included;

<sup>1</sup> WINTERSTEIN: Berichte d. deutsch. chem. Gesellsch., xxviii, p. 167; Zeitschr. für physiol. Chemie, 1894, xxix, p. 521.

the nitrogen probably belongs to the "cellulose" itself. All attempts to separate the nitrogenous constituent from the portion which yields sugar on hydrolysis have failed.

## CRUDE FIBRE.

The percentages are calculated on the dry substance.	Per cent.
Hypholoma candolleum . . . . .	12.1
Clitocybe multiceps . . . . .	9.6
Coprinus atramentarius . . . . .	9.3
Morchella esculenta (young) . . . . .	9.5
" " . . . . .	8.7
Pleurotus ostreatus . . . . .	7.5
Coprinus comatus . . . . .	7.3
Polyporus sulphureus . . . . .	3.0

**Soluble Carbohydrates.**—A considerable portion of the solids of the mushrooms is made up of soluble carbohydrates, while starch is ordinarily not found. Trehalose, a carbohydrate of the formula  $C_{12}H_{22}O_{11}$ , and resembling maltose in some respects, has been isolated from a number of species;<sup>1</sup> and in an extensive series of investigations Bourquelot<sup>2</sup> has described a number of carbohydrates including mannite.

In order to get some idea of the amount of soluble carbohydrates present a number of experiments were carried out in the manner described under the methods of analysis. Since trehalose, for example, cannot be quantitatively converted into dextrose by hydrolysis with acids,<sup>3</sup> the results of analysis must be somewhat low. Nevertheless the data may be of comparative interest as indicating a high content of soluble carbohydrate.

<sup>1</sup> WINTERSTEIN: Zeitschr. für physiol. Chemie, 1894, xix, p. 70. The references to earlier literature are given.

<sup>2</sup> These investigations were published in a series of papers in the Comptes rendus and other scientific journals.

<sup>3</sup> WINTERSTEIN: 1894, *loc. cit.*, xix, p. 77.

DEXTROSE FROM HYDROLYSIS OF WATER-SOLUBLE  
CARBOHYDRATES.

The percentages are calculated on the dry substance.	Per cent.
Pleurotus ostreatus . . . . .	18.6
Coprinus comatus . . . . .	18.0
Morchella esculenta . . . . .	15.3
Polyporus sulphureus . . . . .	12.2

To what extent these soluble carbohydrates are available for absorption in their natural form or after digestion it is impossible at present to say. Such qualitative tests as were made showed them to be transformed to reducing sugars rather slowly by the action of saliva. The large undigested residues (26-59 per cent) found in artificial digestions likewise suggest that they are not completely transformed in the alimentary canal. Reference may here be made to the observations of Stone<sup>1</sup> in feeding experiments on animals. He found that the pentosans, which are so widely distributed in vegetable foods, are to a marked degree less digestible than the carbohydrates, with which they have usually been indiscriminately classed in analyses.

After the presentation of the preceding analytical data it will scarcely be necessary to draw any elaborate comparison between the fungi and other well-known vegetable substances considered as food-stuffs. It may be well to emphasize the deficiencies of the methods commonly followed in estimating the proteid content of vegetable foods, and to call attention to the erroneous inferences which are consequently drawn regarding the nutrient value of these products. Thus it is not unusual in the construction of dietetic tables to multiply the weight of nitrogen obtained by 6.25 and to express the result as "crude proteids."<sup>2</sup> But even where the precaution has been taken to remove non-proteid nitrogenous bodies by extraction with alcohol, the application of the "proteid factor" (6.25) to the N. of the residue may be quite misleading; for our results have demonstrated that the amount of unavailable nitrogenous material — largely, if not entirely,

<sup>1</sup> STONE: American chemical journal, 1894, xiv, p. 13.

<sup>2</sup> Cf. WILEY: Agricultural analysis, 1897, iii, p. 543.

non-proteid in nature — is frequently equivalent to over half of the non-extractive nitrogen present (cf. Table II, p. 232). When it is remembered that the various species of mushrooms examined contain from 75 to 90 per cent of water, the amount of proteid in them appears strikingly small even when calculated on the total nitrogen in the fungi.<sup>1</sup> For example, *Morchella esculenta*, a species of average composition as regards total solids (10.5 per cent) and nitrogenous constituents (0.48 per cent N) could contain as a possible maximum only three per cent of proteid, corresponding in this respect with potatoes, peas, green corn, etc.;<sup>2</sup> the vegetarian would thus be obliged to consume several kilos of the fresh morel to obtain the daily requisite of 100 grams of proteid. The expression "vegetable beef-steak" accordingly seems scarcely appropriate when applied to mushrooms in a strictly chemical sense. Moreover, the comparative poverty of many species in proteids is corroborated by the results of other investigations now in progress in this laboratory, the yield of isolated substance being quite small. The fungi thus form no exception to the ordinary classes of fresh vegetable foods; indeed, they take a decidedly inferior rank in comparison with many.

The carbohydrate content of the fungi is relatively high; but until more is known regarding the nature and digestibility of the carbohydrate constituents of various vegetable foods, it will be useless to draw comparisons. As dietetic accessories the edible fungi may play an important part; but investigation has demonstrated that they cannot be ranked with the essential foods.

<sup>1</sup> Cf. MÖRNER, C. Th. : Zeitschr. für physiol. Chemie, 1886, x, p. 515.

<sup>2</sup> Cf. ATWATER, W. O. : Foods: nutritive value and cost, *loc. cit.*, p. 27.

## THE RESTORATION OF COÖRDINATED, VOLITIONAL MOVEMENT AFTER NERVE "CROSSING."

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A CURSORY glance at a tabulated list of the voluminous literature<sup>1</sup> treating of the union of nerves after division will indicate that many eminent experimental investigators have zealously re-studied and discussed this subject ever since the historical experimental results of Cruickshank<sup>2</sup> were announced.

An analysis of this voluminous physiological and surgical literature fully substantiates the present consensus of opinion that if the cut ends of the central and peripheral portions of a recently divided mixed nerve be brought into apposition, complete restoration of function may ultimately occur in the peripheral portion. Further, the results of a number of physiologists (Flourens,<sup>3</sup> Bidder,<sup>4</sup> Schiff,<sup>5</sup> Philippeau and Vulpian,<sup>6</sup> Reichert,<sup>7</sup> Howell and Huber,<sup>8</sup> and others) indicate that if the central end of one divided mixed nerve be sutured to the peripheral end of another mixed nerve, union of the two ends may ultimately occur, and the function of the nerve fibres of the peripheral portion be re-established. That is to say, the nerve fibres composing the peripheral portion regain their function of conductivity as well as the property of irritability. Degenerated muscle fibres

<sup>1</sup> For the full bibliography of this subject to 1892 the reader is referred to the paper of Howell and Huber, *Journal of physiology*, 1892, xiii, p. 335.

<sup>2</sup> CRUICKSHANK: *Philosophical transactions*, 1795, xvii. See also FONTANA'S description (*Sur le venin de la vipère*, etc., Florence, 1781, p. 177) of Cruickshank's experiments.

<sup>3</sup> FLOURENS: *Recherches expérimentales sur les propriétés et les fonctions du système nerveux*, 1824, p. 272.

<sup>4</sup> BIDDER: *Archiv für Anat., Physiol., und wissenschaftl. Medicin*, 1842, p. 102; *Archiv für Anat. u. Physiol.*, 1865, p. 246.

<sup>5</sup> SCHIFF: *Journal de la physiologie*, 1860, iii, p. 217

<sup>6</sup> PHILIPPEAU and VULPIAN: *Journal de la physiologie*, 1864, vi, p. 421 and 474.

<sup>7</sup> REICHERT, E. T.: *American journal of the medical sciences*, 1885, January.

<sup>8</sup> HOWELL and HUBER: *Journal of physiology*, 1892, xiii, p. 335; 1893, xiv, p. 1.

innervated by the peripheral portion of the nerve also regenerate and again contract to the stimulus of a nerve impulse.

If successful union of crossed nerves can be obtained, it is evident that when the motor nerve (N) of a group of muscles (M) has united to the peripheral portion of a motor nerve (n) of a group of muscles (m), and *vice versa*, the group of muscles (m) must receive their nerve impulses from that group of spinal nerve cells from which the nerve impulses to the group of muscles (M) formerly emanated. Similarly, the cells of origin of the nerve (n) will supply the nerve impulses to the muscles (M). Although a very decided change in the destination of the impulses from the spinal cells of origin of the two nerves has been produced by the crossing, the central relations of those spinal cells with each other, with other groups of cells, and with the neuraxons of the cells of the cerebral cortex have not been anatomically altered by simple division and suture of the peripheral nerve trunks.

Modern histological methods reveal, to some degree at least, how intricate and how wide-reaching are the connections which exist between the various central nervous mechanisms. Naturally, therefore, the old question investigated long ago by Flourens again arises as to whether, or no, the intricately connected central nervous mechanisms are in reality capable of adjusting themselves to the new state of affairs, so that the individual regains complete coördinate control of the muscles supplied by the crossed nerves. Further, if the nerve to a group of muscles which are rhythmically contracting and relaxing in response to rhythmical nerve impulses discharged by a certain group of nerve cells in the medulla or in the spinal cord, be divided and crossed with the central portion of another motor nerve, will the nerve cells from which the axis cylinders of the latter arise ultimately become a rhythmically discharging group and entirely assume the function of the rhythmically discharging cells after the regeneration of the united crossed nerves and muscles?

With a view of obtaining a definite answer to the two preceding questions, the various experiments described in this paper were performed.

**Previous Work on the Subject.** — Although various authors, following in the footsteps of Flourens, have divided and crossed different nerve trunks, motor nerves to supposedly sensory nerves and *vice versa*, and mixed nerves to other mixed nerves, none of these authors has investigated the muscular movements that occur in an animal's leg



with crossed nerves when the motor cortex is electrically or otherwise stimulated. Nor do they appear to have studied the effects upon the rhythmical respiratory and other movements of the vocal cords that may follow the crossing of one recurrens with another motor nerve. Most of the earlier investigators busied themselves with the solution of the problem as to whether, or no, sensory nerves would unite to motor nerves and *vice versa*, with the ultimate re-establishment of the function of the united portions. It is almost needless to point out that the results of such attempts do not come within the scope of this paper. Consequently I confine myself to a brief review of those previous observations that more directly bear upon a part of my own experiments.

According to Flourens'<sup>1</sup> description of his celebrated experiments, the two principal trunks of the brachial plexus in a cock were cut, and the peripheral end of the trunk supplying the upper surface of the wing was sutured to the central end of the other trunk, supplying the lower surface of the wing. At the end of several months the bird had regained perfect use of the extremity of the wing, which no longer dragged, and served for flying (?) as well as before the experiment. When the nerves were exposed, they had completely united in the order in which they had been placed, the inferior end of one nerve being continuous with the superior end of the other, and *vice versa*. In describing the physiological investigation of these united nerves, Flourens writes:<sup>2</sup> "I pinched the nerves above the point of their reunion, — the wing moved at once, and the animal cried; I pinched them below, and the animal felt it as before, and his wing moved again; the same thing took place, when I pinched the enlarged point of reunion. And further, when I pinched the superior nerve above the point of reunion, the muscles of the lower surface of the wing contracted; and, on the contrary, the muscles of the upper surface of the wing contracted when I pinched the inferior nerve, — always above the point of reunion."

<sup>1</sup> FLOURENS: *loc. cit.*

<sup>2</sup> "Je pinçai ces nerfs *au-dessus* du point de leur réunion, l'aile se mut aussitôt, et l'animal cria; je les pinçai *au-dessous*, l'animal le sentit de même, et son aile se mut encore; pareille chose eut lieu, quand je pinçai le *point grossi* de la réunion. Et de plus, quand je pinçais le nerf supérieur *au-dessus du point de la réunion*, c'étaient les muscles de la face inférieure de l'aile qui se contractaient; et c'était, au contraire, les muscles de la face supérieure de l'aile qui se contractaient quand je pinçais le nerf inférieur, toujours *au-dessus du point de la réunion*."

While this result of Flourens appears to be all that one could desire, the observer neglects to state whether the action of the tensors of the patagium, the nerves of which were probably not divided, was taken into consideration and properly excluded. No mention is made of a microscopical, or even of a very careful anatomical examination of the tissue between the necessarily adjacent crossed trunks. If the central and peripheral ends of the nerves in reality united with each other without the formation of a single nerve fibre between the two adjacent points of suture, the fact is all the more remarkable, for no special precautions seem to have been taken at the primary operation to prevent the latter occurrence, although such a new formation will invariably occur, according to the experience of the writer, unless prevented by some such method as is described below.

In other cocks and in a duck, Flourens sutured the central end of the fifth cervical nerve to the peripheral end of the divided vagus, and, after the expiration of a number of months, divided the other vagus. All the birds died in from one to four days after the latter operation. Information regarding the return of irritability to the united nerves is not given, but evidently the vagal functions had not been re-established *via* the nucleus of the fifth cervical nerve.

The experiments on dogs by Philippeau and Vulpian,<sup>1</sup> in which the central end of the vagus was crossed with the peripheral end of the hypoglossal and *vice versa*, only tend to show that mixed nerves of different origin are capable of union, and throw no light upon the positive restitution of voluntary coördinate control of the groups of muscles supplied by the above mentioned nerves. These observers concluded that although the central end of the vagus would unite to the peripheral end of the hypoglossus, the nerve fibres of the peripheral part of the hypoglossus would not recover their connections with their exciting nerve-centre, and the hypoglossus would be but an instrument at the command of the functional centre of the motor fibres contained in the cervical part of the vagus; a conclusion that seems to be substantiated by the results of the later experiments of Reichert.<sup>2</sup>

After suturing in five dogs the central end of one vagus to the peripheral end of the hypoglossus, Reichert found, after the nerves had

<sup>1</sup> PHILIPPEAU and VULPIAN: *Journal de la physiologie*, 1864, p. 421.

<sup>2</sup> REICHERT, E. T.: *American journal of medical sciences*, 1885, January.

united, that certain areas were present in the partially atrophied half of the tongue, which contracted synchronously with inspiration or with expiration, and concluded that the motor fibres of the vagus had actually become united to similar fibres in the trunk of the hypoglossal, and that the hypoglossal fibres conveyed impulses which were peculiar to the vagus apparatus.

Rawa<sup>1</sup> has obtained such remarkably incredible results after crossing nerves of different destination, and also nerves of special function, that one would naturally suspect that his observations and methods must be faulty. For instance, we are told in regard to the cats in which the hypoglossus was sutured to the vagus and *vice versa*, "that of the entire number of cats only six survived. Four of these cats (Nos. 4, 7, 9, 10) had the left central stump of the hypoglossus sutured to the peripheral vagus; two, a similar crossing of the nerves on the right side. In two other cats (12 and 14) the central vagus was sutured to the peripheral hypoglossus on the left side, and in cat No. 16 on the right side." At the expiration of 16-20 months, the right vagus was cut in cats 4 and 7, and both animals promptly died within five days. In cat 12, section of the opposite hypoglossal nerve was followed by loss of power to move the tongue. In cat 16, after the opposite hypoglossal was cut, no movements of the tongue were present, but in a few days the tongue was slowly moved, being contracted to the left, but the animal was killed at the end of six weeks. On page 310, cats 9, 10, and 11 are said to have died very shortly after the primary operation, although cats 9 and 10 were previously included among the six (?) cats that survived the section of one vagus. Likewise, one finds that cat No. 8, previously unincuded in the number of cats surviving the section of the right vagus, was operated on 16-20 months after the primary operation, and two centimetres of the left vagus were excised. Five days later, fearing to lose the animal, it was used for an experiment, for the details of which the reader is referred to the original paper. Rawa's experience leads him to conclude that (1) "after the peripheral portion of a nerve supplying a certain muscle has united to the central end of a nerve that supplies another muscle, the function of the former muscle becomes restored. (2) The direction of the voluntary motor impulses may be altered as one pleases, and the impulses will always accommodate themselves to the peripheral nerve endings." As a result of his experiments in crossing the hypoglossal and vagus, he

<sup>1</sup> RAWA: *Archiv für Physiologie*, 1885, p. 296.

likewise concludes, "that the central nervous mechanisms can innervate organs that formerly did not connect with them, as soon as those organs become connected to them by nervous conductors." "Nerve centres will, by practice, supply exactly what the peripheral organs with which they became connected require of them."

Howell and Huber<sup>1</sup> crossed the ulnar and the median nerves in dogs and succeeded in getting the crossed nerves to unite without the formation of a cicatrix, common to all the ends. They found, to quote these observers verbatim, "that at the second day after the operation, with both median and ulnar cut on the left side high in the arm, and with the ulnar cut on the right side at the level of the elbow, there was very little evidence of any paralysis or even awkwardness." "Before the end of the first week the animal was running around in perfect freedom, and the closest scrutiny could detect no awkwardness of movement except possibly in running rapidly up stairs he would frequently stumble with his front feet; but whether this was due to the unusual innervation of the muscles, or was caused by the over-zealous activity characteristic of young dogs generally, could not be determined." The close relation between the origin and distribution of the median and ulnar nerves led these observers to remark that "a more interesting suture would probably be one between the musculo-spiral and ulnar in which centres of origin of extensor fibres would be obliged to innervate flexor muscles." They considered there was no histological or physiological obstacle to such a union, but considerable awkwardness of movement in the beginning might attend the functional use of the nerve by the animal. Judging, therefore, from the results of Howell and Huber it would appear that such nerves as the ulnar and the median, which innervate in the dog synergic groups of muscles, are not the ones to choose for crossing when it is desired to investigate the return of voluntary coördinated movements in muscles innervated by crossed nerves. The suture of two nerves supplying antagonistic groups of muscles will yield results that can be more accurately interpreted.

From the preceding brief historical review it is evident that there is room for considerable doubt as to whether the central nervous mechanisms concerned in volition and coördination will in reality adjust their nervous discharges so that a grown animal will regain full control of antagonistically acting groups of muscles after their nerve trunks have been crossed.

<sup>1</sup> HOWELL and HUBER : *Journal of physiology*, 1892, xiii, p. 335.

**Methods.**—All the successful experiments were performed upon dogs. In two monkeys which I had hoped would prove more suitable than dogs for this variety of experiment, the ulnar and the median nerves were crossed with the musculo-spiral nerve, but as the experiments were not a success, no further mention need be made of them. Ether anæsthesia was employed for every operation, and all the operations except the last were performed with the strictest aseptic and antiseptic precautions. After the cerebral cortex, etc., had been investigated at the final operation, the animals were killed with an overdose of the anæsthetic.

The nerves were divided with a sharp razor and sutured with fine catgut prepared by the writer's formalin method.<sup>1</sup> Usually two to four fine sutures were employed. After the crossed nerves had been sutured, broad pieces of fascia covering the neighboring pectoral and other muscles were dissected off, and both of the apposed crossed nerves were gently wrapped, for about three-quarters of an inch above and below the point of suture, in separate pieces of this thin tissue, which was then sutured with fine catgut sutures to the fascia of the adjacent muscles. In all the experiments upon the ulnar, median, and musculo-spiral nerves, the common branch from the musculo-cutaneous nerve to the median was entirely excised, its point of origin from the musculo-cutaneous being ligated with a silk ligature. The wound was sutured with No. 2 catgut, dressed with bichloride of mercury gauze, the whole limb wrapped in cotton, bandaged, and, finally, put in plaster of Paris. The plaster not only encased the toes, but also covered the shoulder, and passed around the upper part of the thorax and the lower part of the neck. The fore limb was thus kept perfectly at rest for at least three weeks. The plaster bandage was then removed, to be immediately replaced by a clean one that was allowed to remain on the dog for four to six weeks. If any tendency to ulceration became evident after the removal of the plaster, it was again applied for two to four weeks, or longer, until the vitality of the tissues had sufficiently recovered to resist external sources of irritation. Consequently, by carefully protecting the peripheral parts, the majority of the dogs did not exhibit the ulcerative disturbances that are very liable to occur in the unprotected skin of the wrist, toes, etc., after division of the chief nerve supply of that region.

Previous to the operation, it was found that many of the dogs

<sup>1</sup> CUNNINGHAM: New York medical journal, 1895, April 20.

would give the paw, and some of the remainder were easily taught to do it also; a circumstance that was later of great assistance in judging whether, or no, the recovery of coördinated voluntary control of the muscles concerned in that movement had occurred. Other methods of testing, such as running up a flight of steps, holding a bone after the bandaging of the uninjured foot, etc., were also used. For the electrical investigation, a du Bois induction coil by Reininger, Gebbert, and Schall was employed. The primary circuit of the coil was attached to the mains of the 115-volt illuminating current with a sixteen candle-power lamp in series with the primary of the coil; .5 ampere of current was registered by the ammeter when the hammer was in action. During the electrical examination, insulating rubber was placed under the nerves to prevent the escape of current to neighboring nerves.

After the animals had been killed, very careful dissections of the united nerves were made. In the animals referred to in this paper it was found, unless it is specially mentioned to the contrary, that the crossed nerves had united in the position in which they had been sutured, and that they were not united in a common cicatrix. If the adjacent united nerves were at all firmly adherent, the result was considered questionable, and was thus rejected.

**Experiments.** — I. *Central portion of right ulnar sutured to the peripheral end of the right median; and the central median to the distal ulnar.*

Dog 1.—Operation January 8, 1895. Plaster bandage removed January 12, and wound found to be healing by first intention. On allowing the dog to run about, it did not appear to limp or seem much inconvenienced by the loss of the functional use of the flexors of the right foot and wrist. Careful comparison with the left foot plainly showed that the right wrist was considerably more extended than the left one, and when the dog was standing with this foot resting on the floor, a considerable part of the palmar surface of the metacarpus touched the floor. If both fore-legs were held up, movements of flexion and of extension of the left paw would occur, but the right paw was held in a state of moderate over-extension, the toes being slightly spread apart. When running up a flight of steps, the dog would often stumble, appearing to strike the edge of a step with the over-extended foot. Owing to the development of a few small ulcers on the plantar balls, the plaster bandage was again applied at

the end of a week, over the whole limb and shoulder, with a few turns around the body. In two weeks this plaster was removed, and on February 26 another careful examination of the animal was made. At this date, the over-extension continued. The right forearm was much smaller than the left from atrophy of the flexor muscles. On putting the left paw into a small boot and giving the dog a bone, the bone frequently slipped from under the right paw by which the dog tried to steady it when he attempted to gnaw it. No movements of the flexor muscles could be detected. After anæsthetizing the animal and exposing the crossed nerves, it was found that the central median had apparently united to the distal ulnar as well as could be desired. The bulbous ends of the crossed central ulnar and distal median had separated about three millimetres, but were connected by a delicate grayish thread-like band that was found to consist of new nerve fibres.

Faradic stimulation of the central median above the point of union produced movements in many of the partially exposed muscles innervated by the ulnar, causing ulnar flexion of the wrist and foot. Stimulation of the distal united ulnar three-quarters of an inch below the point of union also produced ulnar flexion, but not until the strength of the current had been considerably increased. Stimulation of the central ulnar with a rather strong current (10 cm.) produced a faint median flexion of the paw. The distal median had not recovered its faradic electrical irritability, and the electrical irritability of the right distal ulnar was much less than that of the left uninjured ulnar.

After exposing the sigmoid gyrus of both cerebral hemispheres, the areas for extension and for flexion of the paw were stimulated after the paw had been flexed and the arm and forearm made immovable by firm fixation;—extension of the paw readily followed the cerebral stimulus. Only a very slight degree of flexion of the paw could be produced by stimulating the fore limb area in the left hemisphere of this dog, although a stimulus sufficiently strong to produce a severe general fit was finally applied. The central ulnar and the central median nerves were then divided above the points of union and stimulated, the results being the same as before their division. The distal median was then divided and the central ulnar stimulated with a strong current; no flexion of the paw was produced. Stimulation of the central median readily produced ulnar flexion.

Dog 2.—Similar to No. 1, but the dog was kept for seventy-five

days. Over-extension of the paw was still present, and the animal was awkward and stumbled when running up the steps. Cutaneous faradic stimulation of the flexors of the paw showed that, although the faradic irritability of those muscles had been nearly recovered, their irritability was less than that of the flexor muscles of the normal left forearm. Faradic stimulation of the exposed united nerves showed that the nerves were irritable both above and below the points of union, and stimulation of the central ulnar produced well-marked contraction of the muscles innervated by the median. Excitation of the central median produced contraction of muscles supplied by the peripheral ulnar which had been crossed with it. Stimulation of the cortical area for flexion of the paw readily produced that movement.

Dog 3.—The right fore limb of this dog was kept for seven weeks in plaster. At the end of fourteen months, a moderate predominance of the extensors over the flexors of the paw was still evident when the animal was carefully examined. The dog also frequently stumbled when attempting to run rapidly up the steps, and though flexor movements of the right paw were plainly to be seen, the movements did not appear to be quite so actively made in the right leg as in the normal left one. Even after this interval of time, the toes of the right foot were still considerably separated when the animal was resting upon that foot. Electrical excitation of the flexor area of the cortex and of the exposed crossed nerves gave results similar to those met with in dog 2, and needs no further comment. The previously atrophied right flexor muscles had evidently nearly completely regenerated, for the forearms of the dog did not perceptibly differ in size nor did the quantitative faradic electrical irritability of the flexor muscles of the forearms differ much.

The preceding results thus corroborate those of previous workers, in that they clearly prove that one mixed nerve may be crossed with and unite with another mixed nerve. They also clearly demonstrate that the peripheral portion of the crossed united nerve recovers its function of conductivity before it recovers the property of electrical irritability. After the nerves have united and the various groups of muscles have regenerated, nervous impulses emanating from the motor cortex of the brain are still capable of causing the cells of the spinal cord from which the central portions of the crossed nerves arise, to discharge impulses that give rise to contractions of the muscles which the crossed nerves supply. But in the dog, as is well



known, the main functional use of the groups of muscles that are supplied by the ulnar and the median nerves is to produce flexion of the foot, the action of the groups of muscles being synergic and also usually synchronous. Consequently, very little, if any, disturbance of voluntary coördinated flexion of the paw would be likely to follow in the dog when the ulnar and the median nerves have been successfully crossed, a conclusion that is fully exemplified by the result obtained in dog No. 3.

II. *Central end of the right musculo-spiral nerve crossed with the distal portions of the ulnar and the median, and vice versa.*

This operation was performed on nine dogs, but in only four dogs were the experiments successful. In one of these four dogs, No. 2, a large, powerful, restless animal, so much swelling and induration of the tissues developed on the dorsal surface of the wrist from constant attempts to walk upon this surface, that it was impossible to definitely judge whether or not the dog was able to voluntarily contract the extensor muscles of the paw. Evidently the animal was not able to extend the paw intentionally, else it would not have continually flexed the foot at each step and come down upon the dorsal surface of the wrist and foot. Subsequent electrical investigation of the nerves and of the cortical centres showed, however, that the crossed nerves had become at least partially united and regenerated, and that they had recovered their conductivity and electrical irritability.

As the experiments on dogs 1, 3, and 4 yielded essentially uniform results, a description of the results obtained in dog 3 will thus apply to dogs 1 and 4.

Dog 3. — Nerves crossed January 20, 1895, and plaster bandage kept on for two weeks. Wound healed by first intention. Plaster reapplied and kept on for four weeks. Muscles of right forearm markedly atrophied and did not respond to cutaneous faradism. In the course of a week, some contraction of the flexors of the paw, which did not fully relax when elbow was extended. The dog continually held the forearm flexed and the foot was not allowed to touch the ground. When given a bone the animal would attempt to steady it in order to gnaw it by resting the outer side of forearm and flexed foot upon the bone, but was not very successful in keeping it firm.

On October 11th, the dog attempted to use the right leg for walking, but whenever he did so, walked on the back of the foot, on the outer

surface of which was a small ulcer. Ether was administered, the crossed nerves exposed, stimulated, and found to have united. Their electrical irritability had been recovered. Many of the flexor and extensor muscles also responded to direct faradization. After closing the wound and applying an antiseptic dressing to it, and also to the ulcer on the foot, the whole limb was put in plaster with the foot extended. At the end of three weeks the plaster was removed, and the wound and the ulcer were found to be healed. From that time until June 29, 1896, the dog was frequently examined, and the muscles stimulated with mild faradic currents, after previously muzzling the dog, which submitted to this treatment without any especial resistance.

On June 29, 1896, the forearms scarcely differed in size. The muscles of the right forearm seemed to be almost completely regenerated. The right paw was held partially flexed, but when it was carefully observed after steadying the forearm at the elbow, alternating movements of flexion or extension of that paw could be readily seen to occur. When the dog was ordered to give this paw, the animal lifted up the forearm, but instead of extending the foot, the latter was very visibly flexed. Every time the dog walked, the right leg was advanced but the paw was quickly flexed. When a bone was given to the dog, after inserting the left foot in a boot and immobilizing the left wrist by means of a small splint, the movement of the muscles of the right forearm appeared to be so extremely incoördinated that the animal finally held the bone by resting the middle of the forearm upon it. Irregular movements of the adductors and abductors of the toes were also noticed. It should be remarked that this dog had exhibited the above movements early in February, 1896, but certainly no improvement in the coördination of the movements had occurred when the above final examination was made.

The dog also seemed to have recovered sensation on all surfaces of the foot, but the various tests with clips, etc., for determining whether, or not, the animal could correctly localize the position of the peripheral stimulus gave such conflicting results that I am not able to give an opinion in regard to this subject. After anæsthetizing the animal, exposing the motor cortex of both hemispheres, and firmly fixing both elbows so as to prevent any movement at the elbow joint, the cortical area of the right hemisphere for flexion of the wrist was stimulated with a minimal current, and then the same strength of stimulus applied to the area for flexion in the left hemisphere. Result: Extension of the right wrist. Stimulation of the

extensor area, a little further forward in the sigmoid gyrus, produced flexion of the left paw. After repeating this several times the musculo-cutaneous nerve was divided, together with the various flexors and extensors of the forearm; the crossed nerves and blood-vessels being carefully dissected away and protected by cotton wet with warm normal saline solution. After firmly fixing the elbow, the cortex was again stimulated; the flexor area giving rise to contraction of the extensor muscles, the extensor area to flexion of the paw, accompanied apparently by extension of the first phalanges when the current was slightly strengthened.

Two minims of the French oil of absinthe were then injected into the jugular vein. In a few minutes the usual absinthe fit occurred. During the tonic fits the left foot was extended and the right flexed. On immediately excising the small area (extensor) of the left hemisphere, which had been electrically determined to produce flexion of the right foot, the right foot became extended. During another fit the flexor area was excised and the exposed extensor muscles of the right foot no longer participated in the fit.

The preceding results thus conclusively show that the spinal nerve cells from which the musculo-spiral and the ulnar and median motor fibres arise still preserve their connections with the cortical motor mechanisms situated in the sigmoid gyrus.

As far as the cortical areas of this region are concerned, there does not seem to be the least ground for stating that these centres readjust themselves to suit the altered innervation of the groups of muscles which the two united crossed nerves supply. Nor did five months' practice seem to enable the adult dog to regain the functional use of the muscles of the forearm and foot, for, as I have previously remarked, very evident and ample volitional, but incoördinated, movements were visible about five months before the dogs were killed, and none of the dogs showed the least improvement in acquiring any better control of the muscles supplied by the crossed nerves.

III. *Will the rhythmic contractions of certain groups of muscles reappear after union of their motor nerve with the central end of a motor nerve to non-rhythmic muscles?*

To investigate this question, the right recurrens was divided in three dogs as low down in the neck as possible. After carefully freeing the long peripheral portion of the recurrens, it was turned upward around the border of the inferior constrictor of the pharynx and sutured to the

central end of the hypoglossal, which had been cut close to the tongue. The central end of the recurrens was ligated with fine silk, turned toward the root of the neck, and sutured with catgut to the adjacent tissue. Before this operation, these dogs barked very frequently, but after the operation the animals were only able to utter an imperfect, hoarse, stridulous growl. The right half of the tongue was paralyzed, and soon became atrophied and fissured. At the expiration of eight months, it was noticed that the atrophic condition of the right half of the tongue was beginning to lessen, except in dog 3, and also that two of the dogs could move the muscles of that half considerably. At this date, when examined under ether, the regenerated muscles of the right half of the tongue readily responded to faradism. At the end of fourteen to fifteen months, the movements of the tongue seemed to be almost completely restored, except in dog 3, in which the right half of the tongue was permanently paralyzed. Fourteen to fifteen months after the primary operation, the dogs were etherized and the trachea divided just below the larynx. After the insertion of a tube with a short rubber pipe attached to facilitate the administration of the anæsthetic, the anterior composite convolution of both hemispheres was exposed and stimulated with an electrode, the points of which were set one millimetre apart. By carefully adjusting the narcosis, using a stimulus just strong enough to cause the vocal cord to nearly approach the middle line, and carefully removing fluid on the convolutions before applying the electrodes, it was perfectly possible to obtain from both hemispheres adduction of the left vocal cord without any accompanying movements of the tongue when the junction of the præcrucial gyrus and the upper extremity of the anterior composite was stimulated.

When carefully observed<sup>1</sup> from below, or from above, through the widely opened mouth, the right vocal cord was seen to be perfectly immovable. In all of the dogs its position seemed to be about midway between adduction and abduction. When the central hypoglossal or the recurrens which had become grafted to it were stimulated, very evident movements of adduction or of abduction would occur. Sometimes the cord would begin to abduct and then suddenly adduct. When the above-mentioned focus in either hemisphere was stimulated, no movement of the right vocal cord followed unless a very strong

<sup>1</sup> In this connection, the writer wishes to thank Professor Frederic S. Lee for his kindness in carefully observing the movements of the vocal cords on various occasions.

current (secondary at 4 cm.) was applied. With this strong stimulus, movements of the tongue and of swallowing also occurred. Minimal stimulation of the left anterior composite gyrus farther back, where it is joined by the supra-sylvian, was followed by bilateral movements of the split tongue, with adduction, or frequently with abduction, of the right vocal cord. The rhythmical movements of the left were not interrupted. Stimulation of the corresponding area of the right hemisphere produced bilateral movements of the tongue with moderate abduction of the right cord. The left cord did not respond. With the coil at 3 cm., adduction of the right cord occurred.

The left recurrens was then divided, in order to stop the respiratory movements of the left cord, and the above-mentioned regions again stimulated. The movements of the right cord accompanying the movements of the tongue were then more striking, but stimulation of the hemispheres at Krause's laryngeal centre did not produce a movement of the right cord, unless, as previously stated, a current sufficiently strong to produce violent efforts of swallowing was employed.

After killing the dog with the anæsthetic, a dissection of the united nerves disclosed the fact that not only had the sutured recurrens united to the central hypoglossal but that from the latter numerous outgrowths had grown to the base of the tongue and had evidently united with the old peripheral hypoglossal stump. The regeneration of the tongue muscles and the return of voluntary control of the tongue was thus readily explained.

A search for the central end of the right recurrens disclosed the small knobbed end of this nerve about in the position in which it had been sutured; it seemed to be attached to the sterno-thyroid muscle. It had clearly not re-established any connection with the laryngeal muscles.

In dog 3, in which the sutured nerves had been rolled up in a piece of fascia, the outgrowths of nerve fibres from the large hypoglossus had not succeeded in reaching the tongue and producing regeneration of its muscles. When the cortex of this dog was stimulated, no movements appeared in the right half of the split tongue. The right vocal cord responded as in dogs 1 and 2, and no respiratory vocal cord movements could be detected after the section of the left recurrens.

Evidently, therefore, the cells of origin of the hypoglossal nerve do not assume the rhythmical functions of the cells of origin of the recurrens when the latter nerve is made to unite to the central portion of

the former. Clearly, the nerve impulses proceeding from certain nerve centres that innervate the muscles supplied by the *recurrens* do not shunt off by new or by old paths to the hypoglossal nucleus, when this nucleus, or a part of it at least, is caused to become the nucleus of the *recurrens*. How much the less likely, therefore, that the hypoglossal nucleus should assume all the functions of the nucleus of the *vagus*, were that nerve united to the hypoglossus.

To conclude, it is evident that in the dog the central portion of one motor nerve may unite with the peripheral portion of another motor nerve; that the cortical representation of the groups of regenerated muscles supplied by the crossed and united distal nerve is the same as the cortical representation of the groups of muscles that were previously innervated by the central portion before its section; that this cortical representation of the groups, after crossing the nerves, differs from that existing before the nerves are crossed, in that the cortical impulses produce incoördinate movements of the muscles supplied by the united crossed nerve. If two motor nerves supplying two groups of synergic muscles, whose action is to produce almost similar simple movements of an articulation, be crossed, the resultant disturbance of the coördinated mobility of those synergic groups is exceedingly slight, as regards the performance of that particular movement. When groups of muscles innervated by the crossed nerves are of widely different functional use, antagonists, etc., the adult animal (dog) does not regain the power of performing intentional coördinated movements with those muscles, although the fibres of the muscles completely regenerate and recover their former irritability.

Crossing the peripheral portion of the motor nerve of rhythmically contracting muscles to the central portion of the motor nerve of non-rhythmic muscles results in the permanent abolition of the rhythmic action of the former muscles.

In view, therefore, of the foregoing results, it is evident that the central nervous mechanisms do not, as Rawa has claimed, adjust their impulses to suit the altered peripheral innervation, and, by practice, supply exactly what is required of them by the peripheral organs with which they become connected.

# PAPAIN-PROTEOLYSIS, WITH SOME OBSERVATIONS ON THE PHYSIOLOGICAL ACTION OF THE PRODUCTS FORMED.<sup>1</sup>

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WHEN papain, the proteolytic enzyme of the papaw plant, was first subjected to careful study by Wurtz and Bouchut,<sup>2</sup> it was compared in its mode of action to trypsin, not alone because it was active in a neutral medium, but especially because of the character of the resultant products. Thus, it was stated that by the vigorous action of papain upon blood-fibrin complete peptonization resulted, with the formation of some leucin in addition. Naturally, at this time (1879) there was no differentiation of proteoses and peptones; hence all that the above statement implied was a conversion of the proteid into a soluble form, precipitable by alcohol and not coagulated by heat nor by acids, although the presence of leucin would certainly suggest the formation of true peptone. Later, Martin<sup>3</sup> pointed out that the enzyme acts vigorously in the presence of sodium carbonate (0.25 per cent) and that as products of digestion there are formed in both neutral and alkaline solutions an intermediate globulin-like body, peptone, leucin, and tyrosin, the last two being formed in small quantity. Here, likewise, the word peptone must be interpreted as meaning simply soluble proteid, and not carrying the distinction which is now known to exist between the proteoses and true peptones. Still later, however, Martin<sup>4</sup> studied the

<sup>1</sup> An abstract of this paper was presented at a meeting of the American Physiological Society held at Washington, May 4, 1897. See *Science*, N. S., v, June 11, 1897, p. 902.

<sup>2</sup> WURTZ and BOUCHUT: Sur le ferment digestif du Carica papaya. *Comptes rendus*, 1879, lxxxix, p. 425. WURTZ: Sur la papaine. *Contribution à l'histoire des ferments solubles*. Ibid., 1880, xc, p. 1379; and xci, p. 787.

<sup>3</sup> MARTIN, S. H. C.: Papain-digestion. *Journal of physiology*, 1884, v, p. 213.

<sup>4</sup> MARTIN: The nature of papain and its action on vegetable proteid. *Journal of physiology*, 1885, vi, p. 337.

action of papain on the several proteids occurring with the enzyme in papaw juice, and found that the globulin present there was converted by the enzyme into an albumose ( $\beta$ -phytalbumose), and that this substance was transformed into a peptone-like body, which in turn was converted into leucin and tyrosin. In this case the peptone-like body referred to was presumably a true peptone in the modern acceptance of the term. Working with a somewhat different preparation of papain, the writer<sup>1</sup> observed incidentally that in the digestion of blood-fibrin and coagulated egg-albumin, deuteroalbumose and true peptone predominated among the soluble products formed; *i. e.* peptone, non-precipitable by saturation with ammonium sulphate. More recently, Osswald<sup>2</sup> has also reported that papain as studied by him, gave rise to the formation of peptone in neutral, alkaline, and acid fluids, but that digestion was most complete and rapid in a hydrochloric acid solution. With regard to the latter part of the statement, we are inclined to believe that with most proteids the solvent action of papain is greatest in the presence of sodium carbonate and bicarbonate, although a mixture containing a very little hydrochloric acid may be more active than a neutral solution of the enzyme. Much depends, however, upon the presence or absence of extraneous matters in the ferment-preparation and on the amount of proteid present by which the presence or absence of *free* acid is determined. This question, however, is foreign to our present subject.

If the solvent action of papain on proteids is really due to conversion of the proteids into soluble albumoses and peptone, then its action must be compared with that of a true digestive enzyme and the process itself accepted as a genuine digestive process. In this connection it will be remembered that the corresponding vegetable enzyme bromelin, the proteolytic ferment of pineapple juice, is a true peptone-forming enzyme.<sup>3</sup> In fact, it resembles trypsin very closely in its ability, under suitable conditions, to transform the proteid undergoing digestion into true peptone. It is, of course, hardly to be expected that these vegetable enzymes will prove to be identi-

<sup>1</sup> CHITTENDEN: Papoid-digestion. Trans. Conn. Acad. Arts and Sciences, 1892, ix, p. 321.

<sup>2</sup> OSSWALD: Untersuchungen über das Papain (Reuss). Münchener med. Wochenschr., 1894, No. 34.

<sup>3</sup> CHITTENDEN: On the proteolytic action of bromelin, the ferment of pineapple juice. Journal of physiology, 1894, xv, p. 249.



cal in every respect with the corresponding enzymes of animal origin. Indeed, we already know that in the action of bromelin there are certain minor differences at least in the primary or side-products formed as compared with those resulting from gastric and pancreatic digestion. There has, however, been no reason for doubting the ability of papain to form true peptone, although it must be admitted that since exact methods of separating albumoses or proteoses from true peptones have come into use, no one, so far as we are aware, has isolated the pure peptone or determined the extent or rate of its formation in papain-digestion. On the contrary, within the last few years, the statement has come from several sources that papain has no power whatever to form peptone; that its solvent or digestive action on proteids is limited to the production of proteoses and that peptone is never formed. Thus, Gordon Sharp<sup>1</sup> states, that on warming coagulated egg-albumin with one-tenth its weight of papain and a hundred volumes of water for eighteen hours, no peptone could be detected either by saturating the digestive mixture with ammonium sulphate and testing the filtrate with the biuret test, or by dialyzing the digestive mixture and testing the diffusate (after one hour!) with phosphotungstic acid and by the biuret test. Albumoses, however, were formed. In a second communication<sup>2</sup> the same writer states that by the action of papain upon egg-albumin and serum-albumin in neutral, acid, and alkaline solutions peptone is never formed. Further, the opinion is expressed that the formation of peptone by papain is, on biological grounds, not to be expected, since the function of the ferment in the plant consists merely in transforming proteids into soluble compounds adapted for circulation through the open vessels, whereas in pepsin-digestion, on the other hand, the products of proteolysis must be adapted for absorption by osmosis prior to their distribution and utilization in the body. Lastly, it may be mentioned that Dott<sup>3</sup> in a comparative study of papain and pepsin has likewise found that the former enzyme, unlike pepsin, is not able to form pep-

<sup>1</sup> SHARP: Papain-digestion: Complete absence of peptone. *Pharm. J. Transact.*, liii, p. 633, Edinburgh; Abstract in *Chemisches Centralblatt*, 1894, i, p. 512.

<sup>2</sup> SHARP: The action of papain upon egg- and serum-albumin in acid and alkaline solution. *Pharm. J. Transact.*, liii, p. 757, Edinburgh; Abstract in *Chemisches Centralblatt*, 1894, i, p. 830.

<sup>3</sup> DOTT: Comparison of the digestive action of papain and pepsin. *Pharm. J. Transact.*, liii, p. 758, Edinburgh; Abstract in *Chemisches Centralblatt*, 1894, i, p. 831.

tone from egg-albumin. If these statements are correct, then, obviously, papain is quite different in its mode of action from other proteolytic enzymes, and the fact, if such it is, should be clearly established. There would seem to be no great difficulty in arriving at a definite conclusion regarding the matter, and the following experiments have been undertaken with a view to throwing some light upon the question.

In a preliminary experiment, coagulated egg-albumin (from a dozen eggs) was mixed with 800 c.c. of 0.2 per cent sodium carbonate solution, 1 gram of commercial papain added, and the mixture, contained in a closed flask with a little thymol, warmed at 40° C. for three days. Further ferment action was then stopped by boiling, the undissolved matter removed by filtration, the filtrate neutralized with acetic acid, filtered from the precipitate which resulted, and further concentrated. From this concentrated fluid the proteoses were precipitated collectively and completely by saturating the fluid while boiling hot with ammonium sulphate, — carrying out the saturation in a neutral, acid, and ammoniacal fluid successively, as recommended by Kühne<sup>1</sup> for the complete separation of proteoses from peptone. On testing this proteose-free filtrate with the biuret test, giving due heed to the necessity of adding sufficient potassium hydroxide to decompose all of the ammonium salt present, an intense reaction for peptone was obtained. Indeed, it was quite evident from the character of the reaction, that a fairly large percentage of true peptone had been formed.

A similar experiment was tried with coagulated blood-fibrin, this form of proteid being warmed at 40° C. for two days with 1 gram of papain in 800 c.c. of 0.4 per cent sodium carbonate, a little thymol being present. On removal of the proteoses with ammonium sulphate, as described above, a strong biuret reaction was obtained in the filtrate, thus showing the formation of true peptone.

Obviously, one possible danger in experiments of this order, where an alkaline fluid containing so much admixed proteid is warmed at 40° C. for two or three days, is bacterial contamination by which putrefaction may be incited. In the two preceding experiments, thymol was made use of to obviate this danger, but in the next experiment chloroform and sodium fluoride were likewise employed, as follows: —

<sup>1</sup> KÜHNE: Erfahrungen über Albumosen und Peptone, *Zeitschrift für Biologie*, 1892, xxix, p. 1.

1	2	3	4
60 grams fibrin <sup>1</sup>	60 grams fibrin	60 grams fibrin	60 grams fibrin
500 c.c. 0.25% Na <sub>2</sub> CO <sub>3</sub>	500 c.c. 0.25% Na <sub>2</sub> CO <sub>3</sub>	500 c.c. 0.25% Na <sub>2</sub> CO <sub>3</sub>	500 c.c. 0.25% Na <sub>2</sub> CO <sub>3</sub>
5 c.c. chloroform	2.5 grams thymol	5.0 grams NaF	5.0 grams NaF
1 gram papain	1 gram papain	1 gram papain	{ 1 gram papain boiled 5 min.

These mixtures were placed in suitably stoppered flasks, shaken thoroughly to insure complete solution of the sodium fluoride, etc., and warmed at 40° C. for twenty hours, with frequent agitation. At the end of the period the mixtures were boiled and filtered, the filtrates neutralized, concentrated, and the proteoses separated as already described by saturation with ammonium sulphate. On testing the filtrates with the biuret test, Nos. 1, 2, and 3 gave a strong reaction for peptone, the reaction in No. 3 being apparently a little the strongest. No. 4, in which the papain was boiled prior to mixing it with the fibrin, gave a purely negative result, thus showing that the peptone reaction in the preceding mixtures could not have come from any admixture contained in the papain itself, nor in the proteid made use of, and that consequently the peptone found must have been formed in some manner during the experiment. Further, this same negative result affords evidence that the peptone detected was not formed by putrefaction; hence it must come from the proteolytic action of the enzyme, which is plainly not hindered by the presence of either chloroform, sodium fluoride, or thymol. Lastly, it should be mentioned that the striking brilliancy of the peptone reactions obtained in Nos. 1-3 precludes the possibility of any other conclusion than that a fairly large proportion of true peptone was formed.

A similar series of experiments was carried out with coagulated egg-albumin, 75 grams of the moist coagulum being used in each mixture, with results wholly in accord with those just described. Further, another series in which fresh, thoroughly washed rabbit's muscle (60 grams in each mixture) was digested gave similar results, the only difference being that in Nos. 1-3 the peptone reaction was even stronger than with the coagulated proteids, as might perhaps be expected owing to the easier digestibility of the former. It is thus quite apparent that papain is a true peptone-forming enzyme, and

<sup>1</sup> Coagulated blood-fibrin.

furthermore is able to exert this action upon various kinds of proteid matter.

What now is the extent to which this formation of peptone may be carried by papain? In the digestion of proteids with pepsin-hydrochloric acid or gastric juice it has been clearly shown that the formation of peptone rarely exceeds 50 per cent; proteoses usually predominate.<sup>1</sup> With alkaline trypsin solution or pancreatic juice, on the other hand, the formation of peptone is much greater, although the hemipeptone formed is eventually broken down by the continued action of the enzyme into amido-acids, etc., leaving only the anti-peptone. If papain is a true peptone-forming enzyme, related more closely to trypsin than to pepsin, it follows that under favorable circumstances it might be expected to produce even more than 50 per cent of peptone. It is not to be understood by this statement that papain can be compared with trypsin in rapidity of action; but merely that of the proteid dissolved by papain, under suitable conditions, full 50 per cent might not unreasonably be looked upon as convertible into true peptone by the continued action of the enzyme. The correctness of this view has been tested by several series of quantitative experiments in which the proportion of proteoses and peptones formed has been determined as accurately as existing methods will allow.

The first experiment of this nature may be described as follows: Coagulated egg-albumin, formed by pouring the whites of eggs into boiling water acidified with acetic acid, was washed thoroughly with water, pressed, and finely divided. The content of dry albumin was then determined in a sampled portion by drying at 110° C., and igniting the residue to obtain the amount of ash. By this method 10 grams of the moist coagulum were found to contain 1.9257 grams of dry proteid. Three digestive mixtures were then prepared, each containing 150 c.c. of 0.25 per cent sodium carbonate saturated with chloroform, 50 grams of the moist coagulated albumin and 0.75 gram of active papain. To obviate any error that might be introduced through the presence of albumose, etc., in the papain, a fourth mixture was prepared similar to the above, except that it contained no albumin. All four mixtures were placed in closely stoppered flasks and transferred to a warm chamber, where they were kept at 38–40° C. for vary-

<sup>1</sup> CHITTENDEN and AMERMAN: A comparison of artificial and natural gastric digestion, together with a study of the diffusibility of proteoses and peptone. *Journal of physiology*, 1893, xiv, p. 483.

ing lengths of time with occasional agitation. One was allowed to digest for 25 hours, the second was interrupted at the end of 51 hours, while the third mixture and likewise the control were continued for 75 hours. Digestion was stopped by heating the mixture to boiling. It will be noticed in these experiments that the proportion of papain employed was quite small, considering the low digestive power of the enzyme.

The mixtures were analyzed as follows: The undissolved residue, made up largely of an insoluble antialbumid-like substance, together with some unaltered proteid, was collected on a weighed filter, washed thoroughly with water and lastly with alcohol, then dried at 110° C. until of constant weight. The filtrate and washings were then neutralized with dilute acid, and the neutralization precipitate so obtained was collected on a weighed filter, washed with water until free from salts, dried, and weighed. To determine the albumoses, the neutral filtrate and washings were concentrated to a small volume and then precipitated while still hot by saturation with pure neutral ammonium sulphate, giving heed to Kühne's latest modifications of the method.<sup>1</sup> The precipitate was filtered by the aid of a hot-water funnel and washed free from peptone with a hot saturated solution of ammonium sulphate.<sup>2</sup> The precipitate, together with the adherent ammonium sulphate, was then washed into a weighed capsule with hot water, the mixture evaporated to dryness, and finally dried in an air-bath at 110° C. until of constant weight. Obviously, the weight so obtained was the combined weight of the albumoses and ammonium sulphate. To ascertain the value of the latter, the mixture was treated with water containing a little hydrochloric acid, the fluid made up to a definite volume, and in an aliquot portion of the latter the sulphuric acid was determined in the usual manner by precipitation with barium chloride. From the weight of barium sulphate thus obtained the amount of ammonium sulphate was calculated and deducted from the combined weight of the albumoses and ammonium salt. The amount of true peptone formed was obtained in this experiment by deducting the combined weight of the antialbumid and undigested residue, neutralization precipitate, and albumoses from the weight of coagulated proteid used, making the necessary corrections for proteoses, etc., in the papain.

The results from this experiment were as follows, expressed in grams: —

<sup>1</sup> *Loc. cit.*

<sup>2</sup> Continued until the filtrate failed to show any biuret reaction.

Period of digestion at 40° C. . . .	25 hours.	51 hours.	75 hours.
Undissolved residue . . . . .	3.4555	3.1626	.... <sup>1</sup>
Neutralization precipitate . . . .	0.1692	0.0920	0.0075
Albumoses . . . . .	2.6167	2.3700	1.2767
	6.2414	5.6246	
Dry proteid used . . . . .	9.6275	9.6275	9.6275
Peptone formed . . . . .	3.3861	4.0029	

Expressed in percentages calculated on the dry proteid used, these figures yield the following results: —

Period of digestion at 40° C. . . .	25 hours.	51 hours.	75 hours.
Undissolved residue . . . . .	35.8	32.8	....
Neutralization precipitate . . . .	1.7	0.9	0.1
Albumoses . . . . .	27.1	24.6	13.3
Peptone . . . . .	35.4	41.7	
	100.0	100.0	

In considering these figures, emphasis is to be laid upon the fact that the large percentage of undissolved residue noted above is by no means composed mainly of unaltered proteid, but is made up to a considerable extent of a peculiar alteration product which seemingly resembles antialbumid, the formation of which must involve a certain amount of energy on the part of the enzyme. Further, it is to be noted that at the end of twenty-five hours digestion, 62.5 per cent of the proteid is converted into albumoses and peptone, while of these soluble products 56.6 per cent is composed of true peptone, the remaining 43.4 per cent being made up mainly of deuteroalbumose. Moreover, it is seen that as the digestion is continued the proportion of albumoses decreases, peptones being correspondingly increased. To be sure, the figures representing the proportions of peptone formed are obtained by difference, but we see no reason why the methods pursued are not capable of yielding results substantially

<sup>1</sup> Lost by an accident.

correct. Moreover, on testing the three ammonium sulphate-saturated filtrates containing the peptone with the biuret test, the intensity of the reactions obtained corresponded exactly with the above data. In this connection it should be mentioned that even under most favorable conditions the formation of amido-acids or other crystalline decomposition products by papain is very slight.

A second series of experiments similar to the above next demand attention because they help make clear possibly why some observers have failed to find evidence of the formation of peptone by papain. Four distinct mixtures were prepared, each containing 150 c.c. of 0.25 per cent sodium carbonate saturated with chloroform, 50 grams of moist coagulated egg-albumin, and 0.5 gram of papain. The fourth mixture, however, differed from the other three in that the papain was boiled with a portion of the fluid prior to mixing it with the albumin. It thus served as a control to check any possible errors that might arise from the action of the alkali alone on the proteid, or from soluble matter contained in the papain. Of greater importance, however, is the fact that the proportion of papain employed in this series of experiments was considerably less than in the previous series; *i. e.*, 0.5 gram instead of 0.75 gram for every 50 grams of proteid. Furthermore, the papain was a different sample, obtained from a different source, and had been tested solely as to its ability to *dissolve* proteid matter.

The several mixtures were kept at 38–40°C. for varying periods of time, one being removed at the end of 25 hours, the second at the end of 48 hours, while the third and fourth were continued for 72 hours. The mixtures were then analyzed as in the preceding case, with the following results expressed in grams: —

Period of digestion at 40° C. . . . .	25 hours.	48 hours.	72 hours.
Undissolved residue . . . . .	3.8354	3.9759	3.7577
Neutralization precipitate . . . . .	0.0405	0.0387	0.0100
Albumoses . . . . .	2.8180	2.3278	2.5176
	6.6939	6.3424	6.2853
Dry proteid used . . . . .	6.8564	6.8564	6.8564
Peptone formed . . . . .	0.1625	0.5140	0.5711

In the control mixture, in which the papain had been boiled before mixing it with the albumin, 72 hours at 40°C. resulted simply in the formation of 0.03 gram of neutralization precipitate and a trace only of albumose. The undissolved residue when dried weighed 6.9301 grams, the plus weight being due to the insoluble matter of the papain. The slight corrections made necessary by these data have been embodied in the above figures.

The percentage results calculated on the dry proteid used are as follows: —

Period of digestion at 40° C. . . .	25 hours.	48 hours.	72 hours.
Undissolved residue . . . . .	55.9	57.9	54.8
Neutralization precipitation . . .	0.6	0.5	0.1
Albumoses . . . . .	41.1	33.9	36.7
Peptone . . . . .	2.4	7.7	8.4
	100.0	100.0	100.0

Here, for some reason, the formation of peptone was comparatively slight. Although the amount of papain employed in each mixture was less than the quantity used in the first series of experiments, the ratio of papain to dry proteid was much the same in the two cases. Evidently, the papain made use of in this last experiment was far less active than the preceding preparation, as shown also by the large percentage of undissolved residue. To be sure, considerable albumose was formed, but the enzyme was so lacking in vigor that extensive proteolysis was impossible, and as a result the formation of peptone progressed very slowly. Still, even under these adverse conditions, some peptone was formed — easily recognizable by the biuret reaction — and the proportion increased slowly with continued digestion. There is therefore even in this experiment no confirmation of the statement that papain is unable to form peptone, but merely a suggestion of the necessity of obtaining an active preparation of the enzyme in order to arrive at a true understanding of its proteolytic power.

In a third series of experiments still another preparation of papain was employed: one which preliminary experimentation showed to be quite active. Each mixture contained 150 c.c. of 0.25 per cent sodium carbonate saturated with chloroform, 50 grams of moist



coagulated egg-albumin, and 0.75 gram of papain. A control mixture of albumin, etc., in which the papain was boiled to destroy its activity, was also included in the series. In this series, however, digestion at 40° C. was continued for longer periods, and it is likewise to be noted that the ratio of dry proteid to the papain employed varied from that in the previous experiments. Further, the method of determining the albumoses and peptone was somewhat different from that previously used. Thus, after separating the undissolved residue and neutralization precipitate, the neutral fluid was concentrated and the albumoses precipitated by saturation of the fluid in the cold with pure zinc sulphate after the method of Bömer.<sup>1</sup> This precipitate was collected on a filter, washed thoroughly with a saturated solution of zinc sulphate, after which it was dissolved in water, the solution made up to a given volume, and the nitrogen determined in a fraction of the fluid by the Kjeldahl method. On multiplying the values so obtained by the factor 6.25 the amounts of albumoses present were calculated. The figures for peptone were obtained by difference, but were verified by determination of the nitrogen in the zinc sulphate-saturated filtrates. This, however, was not easily accomplished owing to the presence of so much zinc sulphate; but on diluting the fluid with water we were able to determine the nitrogen in a small volume of the mixture, using the Kjeldahl method, and on multiplying the nitrogen found by the factor 6.25 we obtained values not greatly at variance with those given by difference. It is needless to say that the control mixture was treated in a similar manner and corrections made for nitrogen introduced with the papain, etc. Following are the results obtained expressed in grams: —

Period of digestion at 40° C.	48 hours.	98 hours.	144 hours.
Undissolved residue . . .	0.9487	1.0569	1.1136
Neutralization precipitate .	0.0542	0.0127	0.0152
Albumoses . . . . .	0.6250	0.6665	0.7506
	1.6279	1.7361	1.8794
Dry proteid used . . . . .	4.4042	4.4042	4.4042
Peptone formed . . . . .	2.7763	2.6681	2.5248

<sup>1</sup> BÖMER: Zinksulfat, ein Fällungsmittel für Albumosen, Zeitschr. f. analyt. Chem., 1895, p. 562.

Expressed in percentages, calculated on the dry proteid used, these figures lead to the following results:—

Period of digestion at 40° C.	48 hours.	98 hours.	144 hours.
Undissolved residue . . .	21.5	24.0	25.2
Neutralization precipitate . .	1.2	0.3	0.3
Albumoses . . . . .	14.2	15.1	17.0
Peptone . . . . .	63.1	60.6	57.5
	100.0	100.0	100.0

Here we have plain evidence again of the ability of papain under suitable conditions to form relatively large quantities of peptone, the latter, in this experiment, being greatly in excess over all the other products combined. It is furthermore evident that in order to bring out the full proteolytic power of the enzyme (assuming an active preparation) it is necessary that the latter be present in fairly large proportion, *i. e.*, as compared with the proteid matter. When this is the case, as in the present experiment, the element of time is of less moment. In other words, when the ratio of enzyme to proteid is suitable, the maximum digestive action under those conditions is reached in 24–48 hours, and longer exposure at 40° C. fails to increase the proportion of peptone formed. In illustration of this point compare the results of the first and third experiments. In conclusion we think it clearly established that papain is not only a peptone-forming enzyme, but that under proper conditions it is able to transform a large proportion of the proteid matter into true peptone. In confirmation of this statement we have been able to prepare and isolate the pure peptone in quantity sufficient to study some of its physiological properties.

#### SOME OBSERVATIONS ON THE PHYSIOLOGICAL ACTION OF THE DEUTEROALBUMOSE AND PEPTONE FORMED BY PAPAIN.

It has been generally believed for some time past that the primary products which result from the proteolytic action of vegetable enzymes, as well as those formed by the action of superheated water, are somewhat different in nature from the corresponding products

formed by pepsin-acid and by trypsin. Thus, Neumeister<sup>1</sup> has shown that if atmid albumin or atmid albumose, *i. e.*, the albumose formed by the action of superheated water on blood-fibrin, is injected directly into the blood of a dog it appears in the urine wholly unaltered. An ordinary albumose, however, *i. e.*, such as is formed by pepsin or trypsin, when introduced into the circulation (of a dog) appears in the urine more or less hydrated.<sup>2</sup> Thus, proto-albumose appears in the urine in part as deuteroalbumose, while if deuteroalbumose is injected into the blood it appears in the urine as peptone. Peptone, on the other hand, is eliminated wholly unchanged. Neumeister<sup>3</sup> also makes the statement that the products which result from the action of papayotin upon albuminous substances are identical with those formed by the action of superheated water. This implies that the so-called atmid products and the papayotin products are alike in their resistance to the action of pepsin,<sup>4</sup> for it is assumed at least that it is the presence of this enzyme in the kidney which leads to the hydration of the ordinary albumoses during their elimination from the body. In the case of rabbits, where pepsin is wanting in the kidney, the injection of albumoses into the blood is followed by their elimination unchanged (Neumeister).

Moreover, there are certain peculiarities in the chemical composition of the atmid bodies,<sup>5</sup> shared to some degree by the proteoses formed by the action of bromelin<sup>6</sup> — the proteolytic enzyme of pineapple juice, — which lends favor to the view that these bodies are not quite identical with the proteoses, etc., formed by animal en-

<sup>1</sup> NEUMEISTER: Ueber die nächste Einwirkung gespannter Wasserdämpfe auf Proteine und über eine Gruppe eigenthümlicher Eiweisskörper und Albumosen. *Zeitschr. f. Biol.*, 1890, xxvi, p. 77.

<sup>2</sup> NEUMEISTER: Ueber die Einführung der Albumosen und Peptone in den Organismus. *Ibid.*, 1888, xxiv, p. 272.

<sup>3</sup> NEUMEISTER: *Ibid.*, xxvi, p. 82.

<sup>4</sup> Since this paper was written, there has appeared an article by E. Salkowski, "Ueber die Einwirkung des überhitzten Wassers auf Eiweiss," *Zeitschr. f. Biol.*, 1897, xxxiv, p. 190 (Jubelband zu Ehren von W. Kühne), in which it is stated that the atmidalbumose formed by him from blood-fibrin was not resistant to the action of either pepsin, trypsin, or bacteria, thus differing widely from Neumeister's product.

<sup>5</sup> CHITTENDEN AND MEARA: A study of the primary products resulting from the action of superheated water on coagulated egg-albumin. *Journal of physiology*, 1894, xv, p. 501.

<sup>6</sup> CHITTENDEN: The proteolytic action of bromelin, the ferment of pineapple juice. *Ibid.*, 1894, xv, p. 249.

zymes. Consequently, it seemed desirable to study with some care the physiological behavior of the albumoses and peptone resulting from papain-digestion with a view to ascertaining what differences of a physiological nature, if any, exist between the latter products and those resulting from animal enzymes.

As has already been pointed out, the soluble products which are formed in the digestion of coagulated egg-albumin with papain are mainly deuteroalbumose and peptone. These were prepared in considerable quantity by digesting the coagulated albumin from four dozen hen's eggs with 9 grams of papain in 2 litres of 0.25 per cent sodium carbonate for 48 hours at 40° C. in the presence of chloroform. The resultant fluid freed from insoluble matter and neutralization precipitate was concentrated to a small volume and the albumoses precipitated by saturation with ammonium sulphate, boiling hot, from a neutral, acid, and alkaline reacting fluid. The precipitate so obtained was dissolved in water, the fluid carefully neutralized, and then dialyzed in running water until wholly free from ammonium sulphate and other salts. The solution was then filtered from a little insoluble matter (heteroalbumose, dysalbumose) concentrated to a small volume, and a portion tested for protoalbumose by saturation of the neutral fluid with rock salt. No precipitate whatever was obtained, consequently the entire volume of fluid was brought to a syrup and the deuteroalbumose precipitated with strong alcohol. After thorough washing with alcohol and ether, the substance was dried at 100° C. making about 20 grams of pure deuteroalbumose.

To obtain the peptone, the ammonium sulphate-saturated filtrate from the albumoses was treated with 50 per cent alcohol, thereby precipitating a large portion of the ammonium salt, while the residual sulphate was removed from the filtrate, after freeing from alcohol, by treatment with barium hydroxide followed by barium carbonate. On evaporating the final filtrate to a syrup and treating with alcohol, the peptone was precipitated more or less gummy, after which it was dehydrated by successive treatments with absolute alcohol and ether, and finally dried at 100° C. About 10 grams of pure peptone were obtained.

**Mode of Experimentation.**—Our study of the physiological action of the deuteroalbumose and peptone formed above was limited to ascertaining their effects on blood-coagulation, their influence on blood-pressure, and their elimination by the kidneys. In all of the experiments dogs were employed, the animals always being anæsthetized.

Most generally this was accomplished by means of a mixture of equal parts of chloroform and ether, although in some of the experiments morphine sulphate was injected hypodermically followed by the administration of chloroform and ether. In the few cases where morphine was used, it was employed in the proportion of 1 centigram of morphine sulphate for each kilo of body-weight.

The albumose or peptone was introduced either into the left femoral vein or into the facial vein through a cannula connected with a burette. The substance, in the proportion of 0.5 gram per kilo of body-weight, was dissolved in 0.7 per cent sodium chloride solution, the volume of the fluid injected ranging from 30 c.c. to 50 c.c. and never exceeding the latter. The fluid was warmed to 40° C.

To observe the rate at which the blood coagulated, portions about 5 c.c. each were withdrawn at stated intervals from the right femoral artery through a cannula inserted in that vessel, the blood being collected in slender test-tubes to observe the time of coagulation. Each time the blood was withdrawn from the artery the first portion passing out was discarded. Moreover, the cannula was removed and cleaned after each withdrawal of blood. Blood-pressure was registered at the carotid artery, or in some instances at the left femoral artery, using a Hürthle spring manometer and a Baltzar kymographion driven at a slow rate.

**Influence on Coagulation of the Blood.** — The effects of deuteroalbumose and peptone on the coagulation of the blood were observed in eight experiments on dogs ranging in weight from 5 to 11.5 kilos. In the first experiment the dosage of albumose was 0.33 gram per kilo of body-weight, but in four other experiments the dosage was increased to 0.5 gram per kilo, which proportion was likewise used in the three experiments with peptone. Following are the results obtained: —

#### FIRST EXPERIMENT.

Dog, 9 kilos.                      3 grams *deuteroalbumose* in 37 c.c. 0.7 per cent NaCl.  
Injection lasted 2 min. 45 sec.

The normal blood coagulated in 3 minutes.<sup>1</sup>

Blood withdrawn 3 minutes after injection of albumose coagulated in 30 min. .					
"	"	9	"	"	25 "
"	"	22	"	"	27 "
"	"	28	"	"	1-2 hours.

<sup>1</sup> The figure given for the coagulation-time of the normal blood is the average of 2-3 determinations.

## SECOND EXPERIMENT.

Dog, 6.5 kilos. 3.25 grams *deuteroalbumose* in 50 c.c. 0.7 per cent NaCl.  
Injection lasted 3 minutes.

The normal blood coagulated in 10 minutes.

Blood withdrawn 1 minute after injection of albumose coagulated in 1 hr. 27 min.

"	"	4	"	"	"	"	1	"	23	"
"	"	8	"	"	"	"	1	"	19	"
"	"	12	"	"	"	"	1	"	15	"
"	"	18	"	"	"	"	1	"	10	"
"	"	29	"	"	"	"	0	"	58	"
"	"	47	"	"	"	"	0	"	40	"
"	"	49	"	"	"	"	0	"	38	"

## THIRD EXPERIMENT.

Bitch, 7.2 kilos. 3.5 grams *deuteroalbumose* in 50 c.c. 0.7 per cent NaCl.  
Injection lasted 1 minute.

The normal blood coagulated in 9 minutes.

Blood withdrawn 2 min. after injection of albumose was uncoagulated at the end of 18 hrs.

"	"	8	"	"	"	"	"	"	"	"
"	"	25	"	"	"	"	"	"	"	"
"	"	46	"	"	"	"	"	"	"	"

## FOURTH EXPERIMENT.

Dog, 7 kilos. 3.5 grams *deuteroalbumose* in 50 c.c. 0.7 per cent NaCl.  
Injection lasted 1 min. 15 sec.

The normal blood coagulated in 3.5 minutes.

Blood withdrawn 6 min. after injection of albumose was uncoagulated at the end of 36 hrs.

"	"	12	"	"	"	"	"	"	"	"
"	"	18	"	"	"	"	"	"	"	"
"	"	24	"	"	"	"	"	"	"	"
"	"	34	"	"	"	"	coagulated within 5½ hrs.			
"	"	42	"	"	"	"	"	"	3	"

## FIFTH EXPERIMENT.

Dog, 11.6 kilos. 5.6 grams *deuteroalbumose* in 40 c.c. 0.7 per cent NaCl.  
Injection lasted 45 seconds.

The normal blood coagulated in 9 minutes.

Blood withdrawn 2 minutes after injection of albumose coagulated in 7½ hours.

"	"	4	"	"	"	"	"	"	"	"
"	"	7	"	"	"	"	"	"	"	"
"	"	12	"	"	"	"	"	2	hrs. 36	min.
"	"	17	"	"	"	"	"	2	"	31
"	"	26	"	"	"	"	"	0	"	42
"	"	36	"	"	"	"	"	0	"	20
"	"	45	"	"	"	"	"	0	"	23
"	"	55	"	"	"	"	"	0	"	13
"	"	65	"	"	"	"	"	0	"	6
"	"	75	"	"	"	"	"	0	"	3
"	"	85	"	"	"	"	"	0	"	2

SIXTH EXPERIMENT.

Bitch, 5 kilos.

3.7 grams *peptone* in 30 c.c. 0.7 per cent NaCl.

Injection lasted 40 seconds.

The normal blood coagulated in 3 minutes.

Blood withdrawn 5 minutes after injection of peptone coagulated in 6 hours.

"	"	9	"	"	"	"	"	"
"	"	15	"	"	"	"	"	"
"	"	24	"	"	"	"	"	1 hour.
"	"	55	"	"	"	"	"	45 minutes.
"	"	65	"	"	"	"	"	40 "
"	"	71	"	"	"	"	"	9 "

SEVENTH EXPERIMENT.

Bitch, 5.5 kilos.

2.75 grams *peptone* in 30 c.c. 0.7 per cent NaCl.

Injection lasted 30 seconds.

The normal blood coagulated in 1.5 minutes.

Blood withdrawn 3 minutes after injection of peptone coagulated in 3-10 hours.

"	"	7	"	"	"	"	"	"
"	"	13	"	"	"	"	"	"
"	"	18	"	"	"	"	"	"
"	"	39	"	"	"	"	"	"
"	"	50	"	"	"	"	"	76 minutes.
"	"	60	"	"	"	"	"	50 "
"	"	80	"	"	"	"	"	30 "
"	"	90	"	"	"	"	"	5 "
"	"	98	"	"	"	"	"	7 "

EIGHTH EXPERIMENT.

Dog, 5 kilos.

Control experiment. 30 c.c. 0.7 per cent NaCl.

Injection lasted 30 seconds.

The normal blood coagulated in 5 minutes.

Blood withdrawn 2 minutes after injection of salt solution coagulated in 3 min.

"	"	6	"	"	"	"	"	4 "
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Fourteen minutes afterwards 3.0 grams *peptone* in 30 c.c. 0.7 per cent NaCl were injected.

Injection lasted 30 seconds.

Blood withdrawn 2 minutes after injection of peptone coagulated in 10-17 hours.

"	"	13	"	"	"	"	"	"
"	"	40	"	"	"	"	"	"
"	"	82	"	"	"	"	"	3 hrs. 16 min.
"	"	95	"	"	"	"	"	0 " 10 "

From these experiments it is very manifest that both deuterioalbumose and peptone, as formed from egg-albumin by papain, have a marked effect upon the coagulation of the blood. With the dosage employed, namely, fifty centigrams per kilo of body-weight, coagulation is retarded for periods ranging from thirty minutes to thirty-six hours.

Further, some of the experiments seemingly suggest that deuteroalbumose is somewhat more effective than pure peptone in retarding coagulation. It is likewise noticeable that this retarding effect upon coagulation is much more striking and also more permanent in some cases than in others, even though the conditions are apparently the same. Thus, in the second and third experiments, in which the dosage of deuteroalbumose per kilo is exactly the same, there is a marked difference in the character of the results, due, however, we believe, to differences in blood-pressure and to consequent differences in the rate of elimination through the kidneys. In connection with this last statement it is to be noted that in many of the experiments, with both albumose and peptone, the period of retardation shows a steady decrease (as in the fifth, seventh, and eighth experiments) until eventually, 50-100 minutes after the injection, the time of coagulation approaches somewhere near that of the normal blood.

What now is to be said regarding the relationship of these bodies in their action on blood-coagulation to the corresponding bodies of animal origin? Obviously, in considering this question little weight can be attached to results obtained with such products as Witte's so-called peptone, since the latter, as is well known, is a mixture of several albumoses with some peptone. Hence, the earlier results obtained with products in which the two classes of substances — albumoses and peptones — were not differentiated have in the present connection only a general interest.<sup>1</sup> Pollitzer,<sup>2</sup> on the other hand, working with the individual albumoses formed by pepsin-acid, found that while all of these substances prevented or delayed the coagulation of the blood, the primary albumoses were most effective, deuteroalbumose least so. Further, amphopeptone led to variable results, frequently wholly negative, while antipeptone as formed by trypsin was almost entirely wanting in any constant effects. Grosjean,<sup>3</sup> however, observed that the peptone formed in gastric digestion does retard coagulation, although its action is less vigorous than that of

<sup>1</sup> SCHMIDT-MÜLHEIM: Beiträge zur Kenntniss des Peptons und seiner physiologischen Bedeutung. Du Bois-Reymond's Archiv f. Physiol., 1880, p. 33; FANO: Das Verhalten des Peptons und Tryptons gegen Blut und Lymphe. *Ibid.*, 1881, p. 277; THOMPSON: Contribution to the physiological effects of 'peptone' when injected into the circulation. Journal of physiology, 1896, xx, p. 455.

<sup>2</sup> POLLITZER: On the physiological action of peptones and albumoses. *Ibid.*, 1886, vii, p. 283.

<sup>3</sup> GROSJEAN: Recherches sur l'action physiologique de la propeptone et de la peptone. Archives de biologie, 1892, xii.



the albumoses. With antipeptone, Spiro and Ellinger<sup>1</sup> found that the effect produced was dependent entirely upon the dosage of peptone employed. Thus, with 0.6 gram per kilo of body-weight coagulation-time was reduced from eight to four minutes, while with 1.1 grams of peptone per kilo the blood was rendered non-coagulable. Lastly, Thompson<sup>2</sup> has reported that antipeptone in doses up to thirty centigrams per kilo tends to hasten the coagulation of the blood, while deutoalbumose sometimes produces a retardation and sometimes a hastening of coagulation, apparently independent of the dosage.<sup>3</sup> It is obvious from these brief statements that any sharp comparison between the digestive products formed by papain and those resulting from the action of pepsin and trypsin is hardly possible. It is, however, seemingly true that the deutoalbumose and peptone resulting from papain-digestion have a greater retarding effect upon blood-coagulation than the corresponding products formed by the animal enzymes. Thus, papain-peptone in doses of 0.5 gram per kilo never failed (in three experiments) to retard coagulation for 3-10 hours, while of antipeptone a dosage of 0.6 gram per kilo accelerates coagulation (Spiro and Ellinger). Further, papain-deutoalbumose in doses of 0.33-0.5 gram per kilo invariably caused marked retardation of coagulation; far beyond anything reported by Thompson with deutoalbumose in doses up to 0.3 gram per kilo. Any attempt at closer comparison in this direction would hardly be justified with our present knowledge. We would call special attention, however, to the tendency manifested in all of our experiments for the effect produced by papain-deutoalbumose and peptone on the blood to pass gradually off, until finally, as in the fifth experiment, the coagulation-time may be considerably shorter than that of the normal blood. We attribute this result solely to the gradual elimination of the proteid, and as the rate of elimination varies with changes in blood-pressure, etc., produced by the substance, it follows that the duration of the effect upon the blood will vary not only with the do-

<sup>1</sup> SPIRO and ELLINGER: Der Antagonismus gerinnungsbefördernder und gerinnungshemmender Stoffe im Blute und die sogenannte Peptonimmunität. *Zeitschr. f. physiol. Chem.*, 1897, xxiii, p. 135.

<sup>2</sup> THOMPSON: The physiological effects of peptone and its precursors when introduced into the circulation. Interim Report of a Committee consisting of Professors Schäfer, Sherrington, Boyce, and Thompson. Report by the Secretary, 1896-97.

<sup>3</sup> See also the papers on peptone and propeptone, by Gley and by Dastre in the *Compt. rend. soc. de biologie*, 1896.

sage given, but also with the period of its detention within the blood current. Lastly, the acceleration of coagulation observed 65-85 minutes after injection of the albumose (Experiment fifth) suggests that small doses of the substance may produce an effect quite the opposite of that produced by a large dose, as observed by Spiro and Ellinger with antipeptone. In conclusion, we see in these results nothing to warrant the assumption that the two papain products are widely different from ordinary digestive products of a like degree of hydration. They certainly do not differ from the corresponding products of pepsin or trypsin digestion more than the latter products differ among themselves. Thus, according to the experiments of Arthus and Huber,<sup>1</sup> of gelatose, 2 grams per kilo of body-weight are required to render the blood of the dog non-coagulable, while of caseose 1.5 grams per kilo are needed; amounts far larger than are required of an albumose formed from either egg-albumin or blood-fibrin. Indeed, we have observed in a single experiment with pure protogelatose that five grams of the substance (dissolved in water) introduced into the facial vein of a dog weighing between three and four kilos, hastened the rate of coagulation.

**Elimination by the Kidneys.** — As already stated, Neumeister has shown that when ordinary albumoses are introduced into the blood of the dog, they are eliminated in the urine more or less hydrated. The atmidalbumoses, on the contrary, he found were eliminated unchanged. What now is the behavior of the deuteroalbumose formed by papain when similarly injected? In the second experiment already detailed, in which a dog of 6.5 kilos was given 3.25 grams of papain-deuteroalbumose by injection into the facial vein, the bladder (empty at the beginning of the experiment) was found one hour after the injection distended with urine. The fluid, amounting to 150 c.c., was removed, filtered, and saturated, boiling hot, with ammonium sulphate. A heavy gummy precipitate resulted, which after being washed with a saturated solution of the ammonium salt, was dissolved in water and tested. It was composed of unaltered deuteroalbumose. On testing the filtrate from the latter precipitate, after again boiling with ammonium sulphate to ensure the complete removal of the deuteroalbumose, an intense biuret reaction was obtained, thus showing plainly the presence of a comparatively large amount of true peptone. In this experi-

<sup>1</sup> ARTHUS and HUBER: Action des injections intraveineuses de produits de digestions peptique et tryptique de la gélatine et du caséum sur la coagulation du sang chez le chien. Arch. de physiol., 1897, 5, viii., p. 857.

ment, therefore, there was marked diuresis, accompanied by a rapid elimination of the deuteroalbumose, but most important of all, a large proportion of the eliminated albumose underwent hydration into true peptone during its transit from the blood to the urine. Such a result as this, however, is not always obtained. Thus, in the third experiment blood-pressure was greatly lowered, and an hour after the injection the bladder contained only a few drops of fluid, with which no distinct reaction for either albumose or peptone could be obtained. In harmony with these two results, the blood in the last experiment drawn 46 minutes after the injection did not coagulate within 18 hours, while in the first experiment, where elimination was comparatively rapid, the blood drawn 49 minutes after the injection coagulated in 38 minutes. Further, in the fifth experiment detailed above, 85 minutes after injection of the albumose, retardation of blood-coagulation was wholly at an end; indeed, coagulation took place more rapidly than prior to the injection. At this time the bladder was found distended with urine, and the latter gave a strong peptone reaction and a fair separation of albumose.<sup>1</sup> In the fourth experiment, there was no marked diuresis, but 76 minutes after the injection the bladder was half full of urine, the latter giving a strong reaction for peptone with only a trace of albumose. Thus, the results obtained in this connection certainly warrant the statement that whenever papain-deuteroalbumose undergoes elimination through the kidneys of the dog, it behaves in the same manner as an ordinary albumose, being transformed in great part into true peptone. It would seem, however, that injection of papain-deuteroalbumose is less liable to produce suspension of the renal secretion than injections of ordinary propeptone.<sup>2</sup>

With papain-peptone the elimination through the kidneys appeared less marked than with the albumose. Still, in all three experiments the bladder was found, 60–100 minutes after the injection, fairly well filled with urine, and on testing the latter a good biuret reaction for peptone was obtained. In no case was there any separation of an albumose precipitate on saturating the fluid with ammonium sulphate.

**Influence on Blood-pressure.**—Upon blood-pressure the albumose and peptone formed by papain from coagulated egg-albumin have

<sup>1</sup> In a recent preliminary communication (Proceed. Physiol. Soc., Nov. 13, 1897), Thompson has likewise reported that Witte's 'peptone' and Grosjean's peptone when injected into the jugular vein of dogs may lead to a marked increase in the quantity of urine accompanied by an excretion of part of the albumoses and peptone injected.

<sup>2</sup> Compare GROSJEAN: *Loc. cit.*

much the same general effect as that produced by ordinary proteoses and peptone. In all of our experiments, in which the pressure was recorded, the dosage of albumose or peptone per kilo was somewhat larger than that ordinarily employed, namely, 0.5 gram. With this dosage, however, there was in nearly every experiment a marked and rapid fall of pressure lasting for about ten minutes. Moreover, the extent of the fall was seemingly influenced somewhat by the rapidity of the injection, a fact which has been commented upon by Thompson.<sup>1</sup> Our experiments also incline us to the belief that the character of the result may be modified somewhat by the personality of the animal, independent of the dosage and the duration of the injection. The experiments in this direction, however, were not intended to be exhaustive, but simply to throw light upon the main problem as to whether the papain products differ radically from ordinary digestive products. In one or two instances the fall of pressure was hardly noticeable, and in these cases the elimination of the albumose through the urine was quite rapid. The two following experiments may be taken as typical of what was generally observed with deutoalbumose.

In a dog weighing 7 kilos, narcotized by chloroform-ether, 3.5 grams of deutoalbumose dissolved in 50 c.c. 0.7 per cent NaCl solution were injected into the right facial vein, the injection lasting 1 min. 15 sec. The blood-pressure was lowered immediately from 160 mm. Hg to about 25 mm. Within four minutes, however, the pressure began to rise gradually but steadily, and in ten minutes from the time of injection was approximately normal again.

A dog of 11.6 kilos, under chloroform-ether narcosis, was treated with 5.6 grams of the albumose by injection into the femoral vein, the injection lasting 45 seconds. Here, the pressure fell from about 150 mm. Hg to 100 mm. within one minute, gradually rising again to the normal in about seven minutes. In this experiment the fall of pressure was preceded by a slight rise amounting to 5 mm. This initial effect of the injection of deutoalbumose upon blood-pressure, *i. e.* a slight rise, is in harmony with the observations of Thompson<sup>2</sup> with Witte's 'peptone.'

Similar experiments with pure papain-peptone gave corresponding results, namely, an immediate and rapid fall of pressure, the latter rising to the normal again in nine to twelve minutes.

<sup>1</sup> THOMPSON: Journal of physiology, 1896, xx, p. 460.

<sup>2</sup> THOMPSON: *loc. cit.*, p. 461.

# THE GASTRIC INVERSION OF CANE-SUGAR BY HYDROCHLORIC ACID.

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IT has been the custom of Voit<sup>1</sup> for the past thirty-five years to demonstrate upon the lecture table that a 0.3 per cent hydrochloric acid solution acting upon cane-sugar at the temperature of the body causes a rapid inversion of the sugar, as may be shown by the acquired power to reduce Fehling's solution. That the stomach itself may cause this inversion has been noted by several authors, Röbner, Claude Bernard,<sup>2</sup> and others. Furthermore Seegen<sup>3</sup> has shown, on feeding cane-sugar to dogs and killing them two or three hours afterwards, that considerable quantities of cane-sugar with invert-sugar are to be found in the stomach, while only invert-sugar can be detected in the intestines. Seegen therefore concluded that the whole inversion of cane-sugar takes place in the stomach. In an experiment performed by one of us (L.)<sup>4</sup> under Prof. Voit's direction, 30 grams of cane-sugar were given to a starving rabbit, and at the end of six and a half hours the animal was killed and the contents of the various intestinal segments analyzed. The figures in grams obtained are here reproduced:

	<i>Cane-Sugar.</i>	<i>Invert-Sugar.</i>
Stomach . . . . .	0.269	2.356
Small Intestines . . . . .	0.002	0.005
Cæcum . . . . .	0.	2.167
Large Intestine . . . . .	0.	0.102

It is seen here, with the exception of a minute quantity in the small intestine quite accountable as within the analytical error limit, that the cane-sugar is exclusively present in the stomach, and accompanying this cane-sugar is found nine times as much invert-sugar.

<sup>1</sup> VOIT: Zeitschr. f. Biologie, 1891, xxviii, p. 268.

<sup>2</sup> For the Literature see VOIT, *loc. cit.*

<sup>3</sup> SEEGEN: Archiv f. d. ges. Physiol., 1887, xl, p. 41.

<sup>4</sup> VOIT: *loc. cit.*, p. 269.

The question to be solved by us was this: Is the acid of the gastric juice a sufficient agent to accomplish such inversion of cane-sugar as takes place in the stomach?

It is known that an enzyme of the intestines acts to convert cane-sugar into invert-sugar. This was shown by Miura<sup>1</sup> to be true of extracts of the mucosa of the small intestine, not only in the case of the rabbit and dog, but also of the mucosa of still-born infants, where presumably neither bacteria nor ferments introduced through the mouth could have been the cause of the inversion. In addition to the very complete literature cited by Miura may be mentioned the work of Mendel,<sup>2</sup> who found that the paralytic secretion from an intestinal fistula in the dog had the power of inverting a cane-sugar solution made antiseptic by one per cent of sodium fluoride. Although the presence of an inverting enzyme for cane-sugar in the small intestines is definitely proven, a similar enzyme within the mucosa of the stomach has not been found. Miura noticed only a very slight effect upon cane-sugar, when he used extracts from the stomachs of dogs, and none at all after using strips and extracts of the stomachs of still-born children. It may be added that Miura finds that the large intestine also has no influence upon the inversion of cane-sugar.

This being the known history of the behavior of saccharose within the digestive tract, our interest was excited to determine more in detail the extent of the action at the body temperature of hydrochloric acid on cane-sugar. The acids used for this purpose were in 0.1, 0.2 and 0.3 per cent solutions, comparable with the acidity of normal gastric juice. These digestions we have carried on for different periods of time.

The pure crystalline cane-sugar used by us showed, after inversion by boiling half an hour with a 0.1 per cent hydrochloric acid and subsequent neutralization, a reducing power equal to 100 per cent of the substance employed. A known quantity of this sugar in solution was brought into one beaker glass, and the necessary amount of dilute hydrochloric acid into another. Both beakers were placed in a thermostat at a temperature of between 37-40°, until they had acquired that temperature. The hydrochloric acid was then poured upon the sugar solution, and at the end of the required time the whole was exactly neutralized with sodium hydrate. As a matter of conven-

<sup>1</sup> MIURA: *Zeitschr. f. Biologie*, 1897, xxxii, p. 266.

<sup>2</sup> MENDEL: *Archiv f. d. ges. Physiol.*, 1896, xliii, p. 434.

ience, we found it best to use as a supply a 0.5 per cent hydrochloric acid solution, and a sodium hydrate solution 1 c.c. of which would exactly neutralize 1 c.c. of the acid. The sugar solutions digested usually approximated one per cent, although five per cent solutions were also used, the total bulk being about 100 c.c. in each experiment. The sugar determinations were made in duplicate, according to the gravimetric method of Allihn. One hundred parts of invert-sugar found were calculated as ninety-five parts of cane-sugar.

The results obtained are given in tabular form below.

TIME.	0.91 % Cane-Sugar.	0.95 % Sugar.	0.91 % Sugar.	5 % Sugar.	0.91 % Sugar.
	0.1 % HCl.	0.2 % HCl.	0.2 % HCl.	0.2 % HCl.	0.3 % HCl.
1 Hour . . .	....	14.0 %	....	15.5 %	22.2 %
2 " . . .	....	25.4	....	29.9	37.6
3 " . . .	....	30.9	....	34.2	49.5
4 " . . .	26.5 %	....	37.8 %	43.0	58.9
5 " . . .	....	....	47.5	59.6	62.4
7 " . . .	{ 40.6 39.3	....	76.8	69.2	79.3
10 " . . .	....	....	81.7	....	93.4
12 " . . .	63.8	....	86.4	....	94.1

The results show that the stronger the acid the greater the inversion. In general the same percentage inversion is obtained with a 5 per cent sugar solution as with a 0.91 per cent solution. The amount of the sugar inverted by the same acid is thus proportional to the strength of the sugar solution. While the acid is acting, the quantity of cane-sugar becomes continuously less in the solution. Hence a smaller amount is continuously being inverted. If we take the case of the 0.91 per cent sugar and the 0.1 per cent acid we find that 26.5 per cent of the sugar is inverted in four hours. If this proportionate decrease be maintained for twelve hours, 60.3 per cent of the sugar should be inverted, whereas, in fact, 63.8 per cent is found so changed. The process involved is dependent on Wilhelmy's law of chemical change.<sup>1</sup>

It seemed important to determine whether hydrochloric acid in

<sup>1</sup> OSTWALD : *Lehrbuch der allgemeinen Chemie*, 1887, ii, p. 617.

chemical combination with proteid and the products of proteid digestion had this power of inverting cane-sugar. It was found that beaten and dialyzed white of egg digested with 0.3 per cent hydrochloric acid and pepsin until no reaction is given with tropæolin has no inverting power upon cane-sugar.<sup>1</sup> The sugar test was made after twice precipitating the proteid with acetic acid and absolute alcohol, in each case neutralizing and evaporating the alcohol. No reduction of Fehling's solution could be obtained.

If we now compare our results with the figures given for the experiment on the living rabbit, we find in the rabbit's stomach after six and a half hours, ten per cent of cane-sugar and ninety per cent of invert-sugar; while, on the other hand, in our beakers using 0.2 per cent and 0.3 per cent hydrochloric acid solutions, were found after seven hours between seventy and eighty per cent of invert-sugar, and twenty to thirty per cent of cane-sugar not inverted. Perhaps the higher inversion in the animal was due to the continual motion in the stomach, or possibly even to an acid of greater strength. At all events the results obtained in the beakers are not very divergent from those found in the animal.

We may perhaps draw the following picture of the fate of cane-sugar within the body. Sugar solutions (dextrose) up to 5 per cent according to Tappeiner<sup>2</sup> and Brandl<sup>3</sup> are not absorbed by the stomachs of dogs. In higher concentrations absorption takes place through the stomach wall, and the mucous membrane becomes flushed with blood and appears very red. If large quantities of cane-sugar be fed to rabbits<sup>4</sup> or to man<sup>5</sup> some of it may appear for a short time in the urine. This cane-sugar absorbed as such we now know from the investigations of F. Voit<sup>6</sup> is not burned in the system, but is quantitatively eliminated in the urine. Since only invert-sugar is to be found in the small intestine, it may safely be argued that the absorption of cane-sugar as such takes place only in the stomach, and there

<sup>1</sup> If unbeaten white of egg be used in the same way, inversion does take place. This we can explain only under the supposition that the digestive liquid penetrates the cells of the egg with difficulty, and that there is therefore free hydrochloric acid present, although on warming in the tropæolin test it has the opportunity of combining with the proteid.

<sup>2</sup> TAPPEINER: *Zeitschr. f. Biologie*, 1880, xvi, p. 506.

<sup>3</sup> BRANDL: *Zeitschr. f. Biologie*, 1892, xxix, p. 287.

<sup>4</sup> VOIT: *loc. cit.*, p. 270.

<sup>5</sup> MORITZ: *Verhandl. d. X Congresses f. innere Medicin*, 1891, pp. 492-501.

<sup>6</sup> VOIT, F.: *Münchener med. Wochenschrift*, 1896, xliii, p. 887.



only when in large quantities. The strongly stimulated gastric mucosa must furnish a gastric juice containing much hydrochloric acid and hence capable of energetic inverting power. The enzyme present in the intestines has the office of quickly transforming into invert-sugar any of the cane-sugar which passes through the pylorus.

Proceeding one step farther, we find the statement of Minkowski<sup>1</sup> that levulose absorbed in pancreas diabetes may be converted into dextrose to the extent of fifty-three per cent. As much as seventy-five per cent of the cane-sugar fed may therefore be converted by the organism into dextrose, the ordinary sugar of the blood.

<sup>1</sup> MINKOWSKI: Archiv f. exper. Pathol. u. Pharmacol., 1893, xxxi, p. 157.



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ON THE MEASUREMENT OF MENTAL ACTIVITY  
THROUGH MUSCULAR ACTIVITY AND THE  
DETERMINATION OF A CONSTANT OF  
ATTENTION.

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IN 1886 Loeb published a preliminary communication in which he showed how muscular force could be used for the measurement of mental activity.<sup>1</sup> He found that the maximum pressure which the flexor muscles of the hand can exercise upon a dynamometer becomes smaller when the person attempts to do mental work simultaneously. The mental work in Loeb's experiments consisted in multiplying and reading. The more difficult the multiplication, the smaller the maximum pressure became. If  $P$  be the maximum pressure without mental work and  $p$  the maximum pressure with simultaneous mental work, the more attention the mental work requires the larger  $P - p$  becomes.

If we assume further that  $P$  is the expression of the attention in the case that the whole available attention is given to muscular work, the expression  $\frac{P-p}{P}$  represents the constant of attention for one kind of mental work. It is of interest to determine how this constant varies quantitatively for different kinds of mental work. At the request of Dr. Loeb, and under his direction, I determined the constant of attention for a number of various mental activities. The experiments were finished during the year 1895. Circumstances, however, have delayed their publication.

<sup>1</sup> LOEB: Muskelthätigkeit als Maass psychischer Thätigkeit. Arch. f. d. ges. Physiol., xxxix, p. 592.

Before I give the results of these experiments it will be necessary to describe the methods used.

# I. ON THE METHODS OF DETERMINING STATIC CONTRACTION.

I used Loeb's dynamograph (Fig. 1), designed for this special investigation. The subject grasps the handle, braces the palm of the hand against the post, then pulls as shown in the drawing.

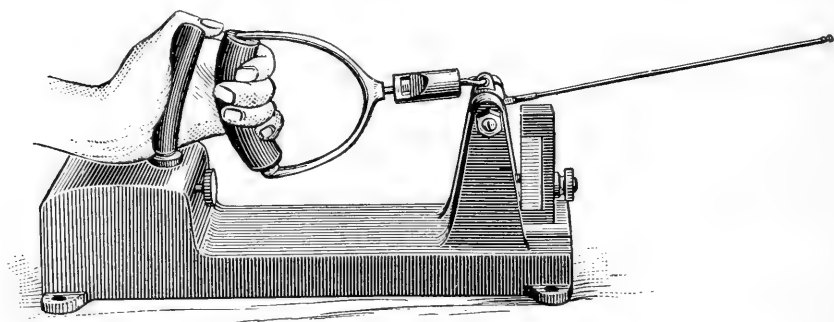


FIGURE 1.

The force of the hand works against the elasticity of a steel bar attached to a movable axle which has a lever. The axle, bar, and lever are all in one piece, which is held by steel screw points. The steel bar rests against a wedge. The length of the part strained can be varied by moving the wedge to any place on the scale.

Fig. 2 is a drawing of the steel wedge and axle. The distance between the handle and the post can be adjusted to hands of different sizes. This distance is kept constant for the same subject. As the bar bends, the axle turns and the lever is raised.

The dynamometer was calibrated by suspending weights to the hooks on the axle, the force being directed by a pulley, in the direction

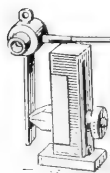


FIGURE 2.

in which the pull is made. The height to which the lever was raised for each additional kilogram was traced on the smoked drum of

a kymograph. In this manner a scale was made by which every kilogram of force applied to the dynamometer could be measured directly.

The static contraction is a contraction caused by a constant innervation. The subject grasped the handle of the dynamometer and gave a constant maximum innervation to the muscles of the hands until the beginning of fatigue was felt, a curve being traced on the revolving cylinder. A time-marker connected with a second's pendulum registered the seconds. The ordinates of the curve represent the force; the abscissæ, the time. The area, or product of the mean ordinate by the number of seconds, expresses the static contraction in terms of kilogram-seconds.

The static work varies as the mean pressure, as the following figures show.

RIGHT HAND.		
Mean ordinate, in kilos.	Number of seconds.	Static contraction in kilogram-seconds.
24.0	34	816.0
16.1	49	738.9
11.3	83	937.9
4.6	170	782.0
3.8	286	1086.0

The abscissæ increase as the ordinates decrease. The maximum static contraction expressed in kilogram-seconds is when the mean ordinate in the series is smallest. Yet the static contraction is greater with the average ordinate at 11.3 than at 4.6 kilograms. So the static contraction is not always greatest with the smallest ordinate. It is a function of both time and force.

The chief source of error in obtaining the maximum constant innervation is lack of attention. The slightest distraction shows a corresponding decrease in the height of the ordinate. With some subjects, the maximum innervation is not given until after several trials, although the subject may think so in the first trial. In all cases the second curve is a little higher than the first. The tonus of the muscle is increased slightly by exercise.

I observed that, whenever the maximum innervation was given to the hand, several groups of muscles were innervated at the same time; as, for example, the muscles of the resting hand, of the face,

TABLE I.

	RIGHT HAND.		LEFT HAND.	
	Innervation of one hand alone.	Innervation of both hands.	Innervation of one hand alone.	Innervation of both hands.
Maximum ordinate . .	27.0 kilos.	25.5 kilos.	25.5 kilos.	26.0 kilos.
	29.0	29.0	24.0	25.5
	26.0	28.0	25.0	25.0
	27.0	27.0	28.0	25.0
	24.0	26.5	23.0	25.0
Mean . . . . .	26.6	27.2	25.1	25.7
Ordinate at 10 seconds	25.0 mm.	24.0 mm.	22.0 mm.	24.0 mm.
	24.5	27.0	21.0	22.5
	26.0	28.0	23.0	24.0
	27.0	26.0	26.0	21.0
	22.0	24.0	22.0	24.0
Mean . . . . .	24.9	25.8	22.8	23.1
Minimum ordinate . .	19.0	22.0	23.0	23.0
	23.0	26.0	22.0	23.0
	23.0	22.5	22.0	20.0
	23.0	25.0	25.0	21.0
	23.0	22.0	21.0	22.0
Mean . . . . .	22.2	23.5	22.8	23.1
Mean ordinate of curve	23.2	23.8	23.5	24.3
	25.5	27.3	22.3	23.6
	24.7	25.1	23.0	22.2
	25.0	26.0	26.3	22.6
	23.0	24.1	22.0	25.5
Mean . . . . .	24.2	25.2	23.4	23.6

and of the jaws. The subject is quite unconscious of this, since his sole purpose is to produce the maximum force. This led me to experiment to find out if the effect would be greater when I gave a maximum innervation to both hands simultaneously than to one alone.

Using the kilogram scale, I measured the ordinates of each curve. I have given, in Table I, four ordinates for each curve, the maximum ordinate, the ordinate at ten seconds, the minimum, and the mean ordinate. The corresponding ordinates in both cases are arranged in parallel columns. In tracing each curve, the handle of the dynamometer was dropped the moment fatigue of the muscles was perceived.

One sees that the corresponding ordinates are usually somewhat larger in the cases in which both hands were innervated at the same time; the difference would have been greater in all probability if in innervating one hand alone I had succeeded in inhibiting all impulses to the other hand.

I repeated the same experiments, varied only by holding a thirty-pound weight in one hand while pulling the dynamometer with the other. The results were practically the same as in the preceding experiments. I give in Table II the mean of each set of ordinates out of five experiments with each hand. The innervations of the pulling hand are more constant when the other hand is holding a weight. This is the only difference in the two series of experiments.

TABLE II.

	RIGHT HAND.		LEFT HAND.	
	Innervation of left hand.	Holding 30 lb. weight.	Innervation of left hand.	Holding 30 lb. weight.
Mean maximum ordinate	26.5 kilos	27 kilos	25 kilos	25.6 kilos
Ordinate at 10 secs. .	26.8 mm.	27 mm.	23.4 mm.	24.3 mm.
Minimum ordinate .	21.2	23.5	21.4	23.3
Mean ordinate . . .	23.7	25.6	22.8	23.8

It seems probable that the effect of the innervation to the hand varies inversely as the number of inhibitions given to the other mus-

cles at the same time. A child innervates several groups of muscles in his first attempts to use one set of muscles. All control seems to depend on inhibition impulses.

## II. DETERMINATION OF THE CONSTANT OF ATTENTION FOR VARIOUS MENTAL ACTIVITIES.

The maximum static innervation of the hand is arbitrarily selected here as a measure of other voluntary activities simultaneously attempted. I do not mean that a muscular equivalent of mental activity can be determined in the sense in which we determine the mechanical equivalent of heat. Loeb found that the maximum force of the hand decreased in proportion to mental work. The decrease of muscular force during mental activity may be due to an act of inhibition of innervations, or may be a result of a division of the constant innervation energy between two simultaneous activities, but in either case the relation of the decrease to the intensity of mental activity is primarily a function of attention. There can, however, be no adequate explanation of attention while so little is known of the physiology of the elements in the nerve centres. Therefore I shall not attempt to explain these facts, but I hope to contribute something to the physiology of attention.

In order to obtain the maximum static contraction curve, the constant undivided attention is necessary, and, if the element of fatigue does not enter in, the innervation will depend upon the concentration of attention. Therefore, according to Loeb, the ratio of the decrease in the maximum constant muscular innervation accompanied by a mental activity, to the original maximum constant innervation alone, gives the constant of attention for any given time. The determination of the constant of attention for various kinds of mental work, affords a method of comparing the degree of concentration of attention required in different kinds of activity.

If  $P$  equals the force of the hand at any given second, in the maximum static curve,  $p$ , the force of the hand at the same time when accompanied by mental activity, then the constant of attention,

$$A = \frac{P-p}{P}.$$

### A. Constant of Attention in Registration of Rhythmical Motions.—

1. In each experiment three curves are traced. Thus, curves (1) and (3) in Fig. 3 are maximum static curves, the former recorded at the beginning, the latter at the end of the experiment. Curve (2) records



the attempt to give a constant muscular innervation to the hand while observing with the eye the vibration of a second's pendulum, and registering each time the bulb reaches its maximum amplitude on either side of the centre. It is at this moment of maximum amplitude that the electric connection is made in the pendulum, and the time-marker records a second. If the tracings of the pen and those of the time-marker coincide, the observations are accurately recorded, showing good attention on the part of the observer. In Fig. 3 (2) the subject begins the mental work first, and then attempts the muscular, while the attention on the observation and registration is continued.

The pneumatic pen of Marey's tambour registered each observation. Two closed rubber tubes connected with the tambour by a

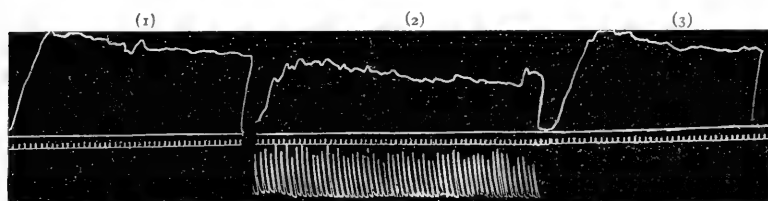


FIGURE 3.

glass T-tube. The pneumatic pen recorded each time the rubber tube was pressed. The lever of the dynamometer, the time-marker, and the pen were arranged in the same vertical line at the beginning of each experiment.

The second maximum static curve (Fig. 3, (3)) was traced to make sure that the decrease in the preceding curve was not due to fatigue. Each curve was continued until the subject was conscious of the beginning of fatigue; a period of rest was then allowed. Every precaution was taken to eliminate fatigue.

I have given in the following tables the results of the last ten experiments with each hand, recorded in Fig. 3. The first experiments gave very irregular results, but after a little practice, I could register observations and pull the dynamometer at the same time much more accurately.

In these tables, four constants, obtained by substituting ordinates, represent the force for the given time. In the formula for the constant of attention,  $A = \frac{P-p}{P}$ ; for the value of  $P$ , the mean of the corresponding ordinates in curves (1) and (3), Fig. 3, is used; for

$p$ , the ordinates of curve (2), corresponding to the same second as those taken in (1) and (3), are employed.

If the attention were constant in each experiment, the ordinate would be the same for each second and the curves would be a straight line. Since there are fluctuations of attention in each experiment I have measured the constant for the beginning, the end, at ten seconds, and for the mean of the ordinates at the several times. The maximum ordinate in curve (2) is often higher because the attention is turned from the mental work when beginning to pull the dynamometer; at the end, the minimum ordinate may be lower than necessary in order to do the registering accurately. The ten-second constant is freer from this error, and, in most cases, is nearer the mean constant.

It should be remarked that where there is inaccuracy in the registration of the vibrations of the pendulum, there is a corresponding increase in the muscular effect. Sometimes, when the subject begins the muscular work after beginning the registration, the lever of the dynamometer will reach nearly its maximum for a second or two, then drop suddenly as the mental work is begun accurately. In beginners in these experiments, there is either a rapid oscillation from one kind of work to another or the innervations given to the registering hand are transmitted to the pulling hand, as the impulses are periodically summed. Probably both these influences are felt.

In Table III (a) I have given the result of ten experiments performed upon myself after I had had some practice in experimenting. Table III (b) gives the results obtained from different subjects.

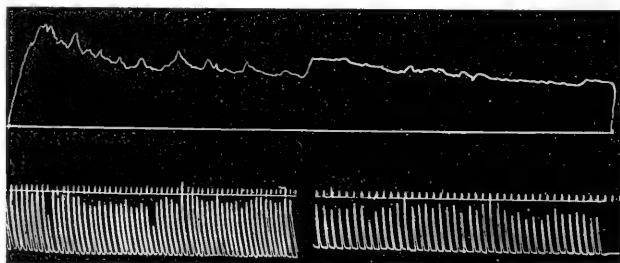


FIGURE 4.

All the curves obtained by the left hand during registration are much more regular than those obtained by the right hand; compare Fig. 4. These irregularities are due, probably, to a summation of stimuli

TABLE III.

(a)

RIGHT HAND.				LEFT HAND.		
	$P.$	$p.$	$A = \frac{P-p}{P}$	$P.$	$p.$	$A = \frac{P-p}{P}$
Ordinates at ten seconds.	30.5	25.0	....	25.0	21.0	....
	32.0	18.0	....	22.5	16.0	....
	25.5	20.0	....	22.0	19.0	....
	30.0	32.0	....	25.0	19.0	....
	25.5	21.0	....	22.7	18.0	....
	25.7	18.0	....	22.5	18.0	....
	28.5	18.0	....	23.0	18.0	....
	24.0	19.0	....	22.0	15.0	....
	26.7	22.0	....	20.7	17.5	....
	28.2	25.0	....	24.7	19.5	....
Mean . . .	27.6	20.8	0.25	22.9	18.1	0.20

(b) Constants obtained from different subjects.

RIGHT HAND.

Subject No.	Max. $A.$	10 sec. $A.$	Min. $A.$	Mean $A.$
2	.09	.12	.33	.32
3	.12	.25	.48	.29
4	.16	.17	.55	.37
5	.13	.35	.53	.34
9	.32	.38	.57	.44

on pressing the rubber tube with the left hand. The stimuli in the rhythmical recording do not pass to the left hand so readily when the right hand is recording, but when the left hand is recording, the rhythmical impulses pass to the right hand as readily as to the left. Physiologically, it is not known why the stimuli seem to pass with

less resistance along the course most used. In one curve (Fig. 5 (2)), the summation is nearly regular for every five seconds. These irregularities are very marked with most beginners. In some cases the hand on the dynamograph follows the rhythm of the pressing of the rubber tube. There was a tendency for the one registering to press harder in the attempt to keep the force of the dynamograph constant. The other subjects always used the right hand, so I think there would be this difference in two hands in most cases. I experimented most with No. 3 (see Table III), nine times—and the ten-second and mean constant of that subject is nearer that of my own. I should expect, however, to find as much difference in this constant for different persons as in the time reaction. I have given one subject the same number in all the series of experiments.

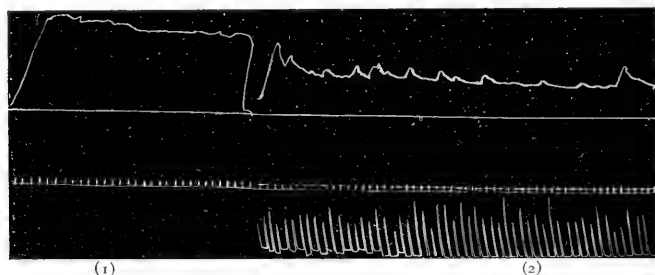


FIGURE 5.

2. The next series of experiments is like the preceding, varied only by pressing alternately two tubes in registering the observations. It required somewhat greater effort of attention in keeping the hand alternating from one tube to the other, and registering at the same time, as shown in curve (2), Fig. 5, when compared with (2) of Fig. 3.

Comparing these constants of attention with the corresponding ones of the first registration, the latter are without exception markedly higher, both in experiments made upon myself and upon others. It may be interesting to note that in spite of the great difference in the maximum ordinate of the six subjects, three of the mean constants are .42, one .41, and one .34.

The same errors, and consequently the same irregularities, appeared in these curves as in the former ones; namely, those irregularities caused by the oscillation of attention from one activity to the other, or by the periodical summation of innervations from the

TABLE IV.

(a) *Registration of vibration by pressing two tubes. Visual observation.*

RIGHT HAND.			LEFT HAND.			
$P.$	$p.$	$A = \frac{P-p}{P}$	$P.$	$p.$	$A = \frac{P-p}{P}$	
Ordinates at ten seconds.	27.2	13.0	....	23.0	13.0	....
	26.0	12.0	....	24.7	15.0	....
	26.5	8.0	....	24.5	18.0	....
	23.5	15.0	....	25.5	14.0	....
	24.5	14.0	....	27.0	15.0	....
	21.5	10.0	....	23.5	14.5	....
	22.5	10.0	....	23.5	11.5	....
	23.5	9.0	....	24.0	17.0	....
	26.5	14.5	....	26.2	15.0	....
	24.2	13.0	....	20.5	13.0	....
Mean. . . 24.3	11.8	0.48	24.2	14.6	0.39	

(b) *Constants obtained from different subjects.*

RIGHT HAND.

Subject. No.	Max. <i>A.</i>	10 sec. <i>A.</i>	Min. <i>A.</i>	Mean <i>A.</i>
2	× .20	.60	.69	.42
3	× .17	.32	.56	.34
4	× .22	.33	.61	.42
5	× .29	.40	.80	.42
9	× .61	.70	.75	.70

rhythmical pressing with the other hand. The latter irregularity was more marked in these experiments than in the first ones. It appeared with the right hand pulling the dynamometer as in the other series. Probably sufficient practice would eliminate these.

3. I determined whether the constants in registering by two

tubes would be less if the observations of the vibrations were made by listening to the ticking of the pendulum rather than by visual observation.

TABLE V.

*Registration by pressing two tubes, auditory observation.*

Ordinate.	RIGHT HAND.			LEFT HAND.		
	$P.$	$p.$	$A = \frac{P-p}{P}.$	$P.$	$p.$	$A = \frac{P-p}{P}.$
Mean Max. .	27.5	20.4	0.26	25.4	17.1	0.35
10 sec. . .	24.2	17.0	0.29	23.5	14.4	0.38
Min. . . .	20.2	14.4	0.28	20.5	11.4	0.44
	Mean $A = 0.28.$			Mean $A = 0.38.$		

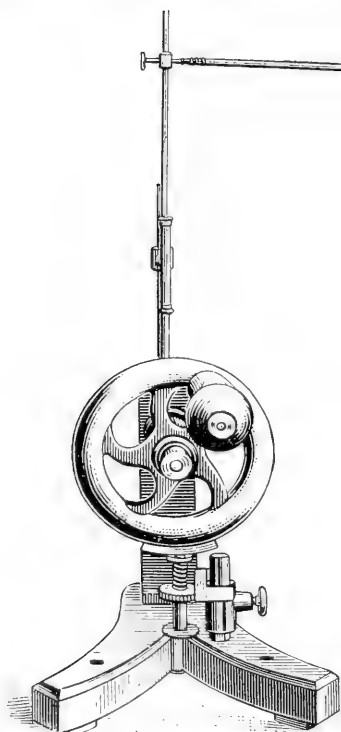


FIGURE 6. Friction Machine.

The constants are less in auditory observation than in visual, when the right hand only is used, but about the same when the left hand is used for the dynamometer. The mean ordinate is .28 with the right hand, and .38 with the left hand. All of the constants of each hand show about this same difference. Most of the constants obtained with the left hand have shown less variation than those of the right hand, and have averaged less in all of the former experiments. Not being able to have subjects for a sufficiently long time to make these experiments with both hands, I cannot say whether this difference in the two hands is common to most subjects, or simply peculiar to the one subject alone.

4. I combined muscular effort with the registration by substituting a friction machine for the tambour. The friction machine (Fig. 6) consists of a wheel and axle to which is attached a jointed vertical rod carrying a long lever. At each revolution of the wheel the rod moves up and down and the lever traces an harmonic curve. The subject turned the wheel one half of a revolution at each observation. Under the wheel is a brake block, which can be lowered or raised by a thumb screw. The brake block is supported on a coarse, stiff, steel spring, and the closer the brake block is pressed against the wheel, the greater the number of units of friction produced between the two through the elasticity of the spring.

I have given the constants obtained by taking the mean of five experiments with each hand when the friction was, 0, 1, 5, and 8, as indicated by the scale beside the brake block

TABLE VI.

*Registration of vibration by friction machine.*

VISUAL OBSERVATION.

I	RIGHT HAND.		LEFT HAND.	
	Mean constant of Max. <i>A.</i>	10 sec. <i>A.</i>	Mean constant of Max. <i>A.</i>	10 sec. <i>A.</i>
F0	.30	.46	.33	.37
F1	.32	.42	.28	.62
F5	.16	.42	.27	.35
F8	.13	.27	.21	.32
	Min. <i>A.</i>	Mean <i>A.</i>	Min. <i>A.</i>	Mean <i>A.</i>
F0	.62	.46	.50	.45
F1	.50	.40	.47	.37
F5	.46	.35	.40	.34
F8	.29	.26	.43	.31

The constants are greater than in the previous experiments when there is no friction, but as *F* increases, the constants decrease. This

decrease in the constants depends upon the increase in the innervation of the recording hand in order to overcome the resistance of the wheel. This is the same phenomenon mentioned before: namely, that the effect in the hand pulling the dynamometer is greater if the same muscles in the other hand are innervated at the same time. Therefore, in  $A = \frac{P-p}{P}$ ,  $p$  increases with  $F$ ; hence  $A$  decreases when  $F$  increases.

One observes the same difference in the constants of the two hands even in a more marked degree in this set of experiments. The impulses passing to the left hand in overcoming friction in turning the wheel affect the right hand more while that hand is pulling the dynamograph than when the left hand is pulling. Therefore the constants for the right hand are much less than those for the left.

**B. Constant of Attention in the Observation of Coincidence of Two Vibrating Pendulums.** — The second pendulum and the pendulum of a metronome were set vibrating in different periods of time. A lever recorded the vibration of the metronome. The metronome pendulum was lengthened and marked with red, and so placed in relation to the second pendulum that the tracings of both would show coincidence at the same time that the coincidence could be seen by the observer. When the subject observed the coincidence he pressed a rubber tube of Marey's tambour. All the levers being arranged in the same vertical line at the beginning, the tracings show whether the observations were accurate.

I give the results of three sets of experiments made in different ways: (*a*) the observation was made by the eye directly; (*b*) the subject looked through a small dark tube which shut off most of the visual field except at the place of coincidence; (*c*) every fifth vibration of the metronome, and every second vibration of the second pendulum were registered. Five experiments were made with both hands in each case.

I found it very difficult to register accurately and to pull the dynamometer even after some practice; none of the first (*a*) series was accurate. Most of the next series (*b*) were nearly accurate. It was easier to see the coincidence through the tube, although it required greater effort in accommodating the eye to the tube. Probably this may be the reason that the constants are somewhat higher in (*b*).



In the last series (c) the mental effort of memory, as well as that of observation, was involved for the first time. It required the greatest effort of attention to remember the counts of both pendulums and do any muscular work at all. In nearly all the experiments the vibration of one of the pendulums would be registered accurately, and that of the other registered irregularly. There was more regularity in the form of the curves and less difference in the results of the two hands in all of these experiments than in the former ones.

TABLE VII.

*Constants of attention in registration of coincidence of two vibrating pendulums.*

(a)	RIGHT HAND.			LEFT HAND.		
Mean Or.	$P.$	$p.$	$A = \frac{P-p}{P}.$	$P.$	$p.$	$A = \frac{P-p}{P}.$
Max. . . .	26.1	20.3	.21	23.6	17.8	.24
10 sec. . .	24.1	16.8	.30	22.3	14.0	.37
Min. . . .	21.4	10.4	.51	19.9	10.3	.51
	Mean $A = .34$			Mean $A = .35$		
(b) <i>Observation through a tube.</i>						
Max. . . .	26.3	17.6	.33	22.4	14.6	.34
10 sec. . .	23.5	15.3	.34	20.9	12.2	.41
Min. . . .	22.9	9.8	.57	19.0	9.8	.48
	Mean $A = .41$			Mean $A = .42$		
(c) <i>Registration of 5th vibration of metronome and 2d vibration of pendulum.</i>						
Max. . . .	25.7	13.2	.48	22.7	11.8	.48
10 sec. . .	24.9	9.6	.61	21.7	9.4	.56
Min. . . .	19.2	6.6	.65	17.9	6.2	.65
Mean. . .	22.0	8.9	.59	20.0	8.3	.58

C. **Determination of the Constant of Attention in Reckoning.** — When reckoning, I found myself whispering each step in the process, and the harder I pulled the dynamometer, the greater was the tendency to whisper, and the greater the effort to remember. The whispering and the writing of the results produced great irregularities in the curves. I finally obtained constants from two series of five experiments each, for both hands. In the first (*a*) I performed multiplication of the numbers from 12 to 20, as  $14 \times 13$ ,  $14 \times 15$ , etc., in every combination, until I was conscious of the beginning of fatigue. The second series (*b*) was done in the same way, with numbers from 20 to 30. In both series, the eyes were closed, there was no whispering, and the results were recorded from memory after each experiment. Then I tried to eliminate all the effects from other muscular impulses, such as whispering, writing, and visual perception.

The constants in (*c*) were obtained in the same way as those of (*a*) and (*b*), by multiplying numbers from 60 to 70; also those in (*d*) by simply adding by 12's, 9's, 8's, etc.

I found it required less effort in reckoning if the figures were before the eye, and the constants in (*c*) were obtained in that way. I could not repeat the reckoning in (*a*) or (*b*), since the practice with those numbers had made it less difficult, so I took the numbers from 30 to 40.

The constants in (*b*) are, on the whole, greater than those in (*a*), especially the mean constant. In the other series they are smaller, although the numbers are larger.

The constants obtained in adding (*d*) are considerably smaller, as we should expect, as the mental effort is less.

Although it seemed much easier to reckon with the eye upon the figures and to repeat each process to myself, the constants obtained with the right hand are about the same as those in (*a*), and less with the left. The numbers were larger (30-40) but the mental effort was about the same. The reason the constants with the left hand are smaller in this particular case I cannot explain. In reckoning all of the other series the mean constants obtained with the left hand were a little higher than those obtained with the right hand. The innervation of the vocal cords and muscles used in speech must have interfered with the innervations of the left hand.

The effort in memory is less with the figures before the eyes, yet the attention in observation is another factor which has a much greater effect on attention than one realizes.

TABLE VIII.  
CONSTANT OF ATTENTION IN RECKONING.

(a) Multiplication of numbers from 11 to 20 with eyes closed.						
	RIGHT HAND.			LEFT HAND.		
Mean ordinate.	$P.$	$p.$	$A = \frac{P-p}{P}.$	$P.$	$p.$	$A = \frac{P-p}{P}.$
Max. . . .	25.3	11.9	.52	23.2	10.2	.56
10 sec. . .	22.9	10.2	.55	21.6	9.3	.56
Min. . . .	19.9	8.8	.55	18.3	7.4	.59
Mean . . .	22.0	10.0	.54	20.4	9.0	.55
	Four errors out of 22 problems.			Four errors out of 18 problems.		
(b) Multiplication of numbers from 20 to 30, eyes closed.						
Max. . . .	26.1	12.1	.53	23.7	10.8	.54
10 sec. . .	23.5	9.4	.60	21.9	9.8	.55
Min. . . .	20.5	7.9	.60	18.4	6.7	.63
Mean . . .	23.2	9.1	.59	20.5	7.9	.61
	Four errors out of 20 problems.			Six errors out of 20 problems.		
(c) Multiplication of numbers from 30 to 40. Results registered. Eyes follow the figures.						
	Max. $A.$	10 sec. $A.$	Min. $A.$	Mean $A.$		
Right Hand . .	.50	.54	.63	.54		
Left Hand . . .	.46	.35	.43	.38		
(d) Adding by 12's, 8's, 9's, etc., eyes closed.						
	Max. $A.$	10 sec. $A.$	Min. $A.$	Min. $A.$		
Right Hand . .	.40	.44	.32	.39		
Left Hand . . .	.46	.48	.46	.47		

<i>(e) Multiplication of numbers from 11 to 20.</i>					
Subject No.	Ratio of error.	Max. <i>A.</i>	10 sec. <i>A.</i>	Min. <i>A.</i>	Mean <i>A.</i>
2	$\frac{3}{4}$	.24	.31	.41	.30
3	....	.65	.65	.62	.64
6	$\frac{1}{2}$	.20	.20	.22	.23
8	$\frac{1}{2}$	.13	.20	.27	.20
10	....	.40	.43	.48	.44
<i>(f) Multiplication of numbers from 60 to 70.</i>					
Right Hand .	Max. <i>A.</i> .46	10 sec. <i>A.</i> .48	Min. <i>A.</i> .46	Mean <i>A.</i> .47	

**D. Constant of Attention in Reading and Writing.**—In this series I determined the constants with the right hand alone. I read an article not very difficult to comprehend from a sociological journal, and determined the constants as before. The following table (*a*) shows that the constants are smaller than those in reckoning.

TABLE IX.

*(a)*

	Max. <i>A.</i>	10 sec. <i>A.</i>	Min. <i>A.</i>	Mean <i>A.</i>
Right Hand . . .	.34	.30	.36	.34
" " . . .	.38	.37	.30	.38

In (*b*) are seen the results of a series of experiments where I used all those coördinated muscles necessary in writing without doing other mental work. I made short straight lines, one after another, all the time I was pulling the dynamometer. Thus I determined the constants for those complex coördinated motions.

This activity requires about the same degree of attention as that in the other two series. The constants for the right hand may be a little higher on account of the greater effort in writing with the left hand. I observe here, that the greater the muscular effect in pulling the dynamometer, the longer the marks, and the harder the other

hand would press on the paper in writing. Accordingly the more the subject inhibited such impulses and wrote naturally, the less the muscular work done. I believe this explains the lower constants of attention obtained from other subjects in this series of experiments(c).

(b) *Constants in writing.*

	Max. <i>A.</i>	10 sec. <i>A.</i>	Min. <i>A.</i>	Mean <i>A.</i>
Right Hand . .	.34	.41	.40	.35
" " . .	.36	.30	.36	.33

(c)

Subj. No.	Max. <i>A.</i>	10 sec. <i>A.</i>	Min. <i>A.</i>	Mean <i>A.</i>
2	.20	.33	.33	.22
3	.28	.25	.31	.24
11	.08 ×	.25	.46	.24

**E. Constants Varied by Beginning Muscular Activity First.**—In all the former determinations, the muscular activity was begun while the mental activity was going on. By reversing this order I found as Loeb had done before that in most cases the corresponding constants were markedly less. The difference is more marked in some subjects than in others, but in all cases the mean constant is less if determined by beginning the muscular activity first.

As I mentioned before, among the sources of errors, a subject often transfers most of the attention from the mental to the muscular work when he first begins to pull the dynamometer, and this is the reason the maximum constants are not more accurate.

The effect of the order of the two activities on the constants of attention may have some theoretical importance. The following tables give the constants side by side, obtained under each condition. The inhibitory effect of the mental activity upon the muscular work is less when the muscular innervation is begun first. Hence the constant of attention is much less for the same activity, as the table shows.

TABLE X.

<i>(a) Registration of vibration of pendulum, 2 tubes.</i>						
	Muscular act. 1st. 10 sec. <i>A.</i>	Mental act. 1st. 10 sec. <i>A.</i>	Muscular act. 1st. Min. <i>A.</i>	Mental act. 1st. Min. <i>A.</i>	Muscular act. 1st. Mean <i>A.</i>	Mental act. 1st. Mean <i>A.</i>
R. H. .	.22	.48	.33	.49	.23	.41
L. H. .	.21	.39	.42	.42	.30	.34
<i>(b) Observation of coincidence of two pendulums.</i>						
R. H. .	.31	.34	.38	.57	.24	.41
L. H. .	.11	.41	.37	.48	.31	.42
<i>(c) Reckoning.</i>						
R. H. .	.26	.55	.56	.55	.42	.54
L. H. .	.13	.55	.14	.63	.39	.61
<i>(d) Writing.</i>						
R. H. .	.10	.41	.13	.40	.01	.35
L. H. .	.25	.30	.05	.36	.14	.33
<i>(e) Registration of vibration of pendulum.</i>						
Subj. No.	Muscular act. 1st. 10 sec. <i>A.</i>	Mental act. 1st. 10 sec. <i>A.</i>	Muscular act. 1st. Min. <i>A.</i>	Mental act. 1st. Min. <i>A.</i>	Muscular act. 1st. Mean <i>A.</i>	Mental act. 1st. Mean <i>A.</i>
R. II. 3	.34	.34	.16	.32	.56	.56
L. II. 4	.27	.42	.10	.33	.44	.61
<i>(f) Reckoning.</i>						
R. H. 2	.28	.30	.20	.31	.16	.41
" " 3	.10	.64	.06	.62	.25	.64
L. H. 8	.01	.20	.06	.20	.14	.27

TABLE XI.

*Fatigue curves.*

	Muscular activity alone.			(a) Muscular and mental activity. Registration of rhythms.		
	Mean or.	No. sec.	Kilo. sec.	Mean or.	No. sec.	Kilo. sec.
Right Hand .	K 21.2	79	1674.8	10.0	157	1570.0
“ “	15.2	72	1049.4	8.0	182	1456.0
“ “	18.4	101	1858.4	13.1	114	1493.4
“ “	22.3	63	1398.6	125.0	86	1075.0
“ “	24.0	13	312.0	15.0	114	1710.0
Left Hand .	21.5	77	1655.5	11.7	192	2246.4
“ “	17.6	76	1337.6	12.9	212	2734.8
“ “	16.5	71	1171.5	11.1	116	1287.6
“ “	15.5	72	1116.0	6.7	274	1835.8
“ “	....	....	....	13.4	146	1956.4
				(b) Reckoning.		
Right Hand .	12.0	130	1560.0	10.5	229	2404.5
“ “	14.5	68	986.0	8.3	298	2473.4
“ “	16.0	115	1840.0	7.4	285	2109.0
“ “	17.5	107	1872.5	6.0	252	1512.0
“ “	7.6	228	1732.8	....	....	....
Left Hand .	10.4	101	1050.4	9.4	303	2848.0
“ “	10.8	202	2181.6	9.0	298	2682.0
“ “	16.0	76	1216.0	8.1	270	2187.0
“ “	8.6	206	1771.6	....	....	....
“ “	14.8	88	1302.4	....	....	....

## III. FATIGUE.

Fatigue entered incidentally into these experiments, and I will give some facts observed. It was found without exception that the feeling of muscular fatigue sets in much later when the muscular activity is accompanied by mental activity. The difference in time was from 15 to 33 per cent.

Other experiments were conducted in the manner just described, but the subject continued the work in all cases until fatigued, instead of stopping the moment fatigue began to be felt.

Table XI gives the static work alone and when accompanied by mental work, in kilogram-seconds.

The tables show that in twelve cases out of fifteen, the static contraction in kilogram-seconds is greater during mental activity. Since the work done in each case is proportional to the area of the curve, the actual muscular work done is the greater when accompanied by mental activity. There are two probable causes for this: (1), a lower mean ordinate; (2), a higher threshold value for the sensation of fatigue during mental work than during muscular activity alone. It is a matter of common experience that one does not feel physical fatigue or any pain so soon when the attention is given to some one activity.

The increase in the threshold value for the perception of fatigue would take place whether the origin of fatigue was in the periphery or in the central nervous system. If the energy of all the innervation from the centres has one common source, the origin of fatigue, in these experiments, must be in the periphery; otherwise the mental work would be done at the expense of the muscular work; but the origin of fatigue is not in the loss of innervation energy in the centre, but in the muscle tissue itself.

## IV. CONCLUSION.

I will make a brief summary of the quantitative results of my investigations by giving a comparative table of the constants of various activities arranged in the order of the magnitude of the mean constants. A study of the tables shows that the constant of attention for any activity increases with —

1. Effort of accommodation of the special sense organs.
2. Effort in coördination of the muscles.
3. Effort of the memory.
4. Number of simultaneous activities.



CONSTANTS OF ATTENTION FOR VARIOUS MENTAL ACTIVITIES FROM ONE SUBJECT.

	R. H.	L. H.
Registration of Vibration of Pend. by one Tube; Visual Per. . .	.23	.21
“ “ “ “ “ two Tubes; Auditory Per. . .	.28	.38
Direct Observation of Coincidence of two Pend. and Register . .	.34	.35
Reading . . . . .	.34	. .
Writing . . . . .	.35	.33
Strained Visual Perception . . . . .	.38	. .
Adding Numbers . . . . .	.39	. .
Observation of Coincidence of two Pend. through Tube and Register .	.41	.42
Registration of Vibration of two Pend. by two Tubes (Visual) . .	.46	.34
Rhythmical Rotation of Wheel with Pend. . . . .	.46	.45
Multiplication of Numbers from 60 to 70 . . . . .	.47	. .
“ “ “ 12 to 20 . . . . .	.54	.55
“ “ “ 20 to 30 . . . . .	.59	.61
Counting of Register of 5th Vibration of Metronome and 2d Vibration of Pend. . . . .	.59	.58

MEAN CONSTANTS OF ATTENTION FROM VARIOUS SUBJECTS.

Subject.	Register of vibrations of pend., one tube.	Register, two tubes.	Writing.	Visual perception.	Auditory perception.	Reading.	Repeating poetry.	Adding.	Multiplying numbers from 18 to 20.	Observation of coincidence of two pend.
2	.32	.42	.22	....	....	....	....	....	.36	. . .
3	.29	.34	.24	.28	.30	.50	....	....	.64	.19
4	.37	.42	....	....	....	.38	....	....	....	.55
5	.34	.42	....	....	....	....	....	....	....	....
6	....	....	....	....	....	....	.09	....	.23	....
7	....	....	....	....	....	.27	....	.39	....	.51
8	....	....	....	....	....	....	....	....	.28	....
9	.44	.70	....	....	....	....	....	....	....	....
10	....	....	.44	....	....	....	.46	....	.44	.10
11	....	....	.24	.09	.04	....	....	....	....	....

From a study of the curves for the two hands, I must conclude that the innervations to perform rhythmical contractions given to the left

hand, while the right hand is innervated to pull the dynamometer, are communicated to the right hand and influence the form of the curve, while innervations to perform rhythmical contractions given to the right hand, while the left is pulling the dynamometer, are not transmitted, or, at least, are transmitted much less markedly to the left hand. Whatever be the physiological explanation, frequent repetition eliminates this difference in the two hands.

After a certain amount of practice, and with care to have like conditions in every case, I believe that the mean constant of attention for any mental activity can be determined for every subject with as slight variations as the personal equation in time reaction.

In conclusion, I wish to express my gratitude to Professor Loeb, for the kind assistance and the many valuable suggestions that I have received from him during the course of my work. My thanks are also due to a number of my fellow students who served as subjects for experiment.

## THE INFLUENCE OF BILE AND BILE SALTS ON PANCREATIC PROTEOLYSIS.

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THE natural commingling of bile and pancreatic juice in the duodenum is strongly suggestive of harmony of action, and it might reasonably be assumed that in pancreatic proteolysis the presence of bile would be in no wise inimical. Indeed, such few observations as have been recorded tend to show, as a rule, that the proteolytic action of the pancreatic enzyme is not materially impeded by the presence of bile or its constituent salts. Thus, many years ago Heidenhain<sup>1</sup> observed that when an aqueous solution of dried pig's bile was added to a glycerin extract of the pancreas, the proteolytic power of the latter was not diminished, but apparently increased. A similar stimulating effect was observed on addition of a 1 per cent solution of sodium glycocholate to the enzyme-containing solution. The few experiments then made were purely qualitative ones, proteolytic power being determined simply by noting the rate at which flocks of fibrin were dissolved. The results, however, were sufficiently convincing to lead Heidenhain to the conclusion that "the salts dissolved in the bile have an influence similar to that of sodium chloride." Some years later Lindberger<sup>2</sup> found that the well known inhibitory action of organic acids upon trypsin-proteolysis may be overcome, to some extent at least, by the presence of bile salts. Thus he observed that the presence of 1-2 per cent of bile with some sodium chloride would enable a trypsin-solution containing 0.02 per cent of lactic acid to digest fibrin as rapidly as a neutral solution of the enzyme; indeed, as rapidly as an alkaline solution, provided the content of alkali was not too great. If, however, the proportion of lactic acid was raised to 0.05 per cent, then bile and sodium chloride were without avail in stimulating proteolysis. Experiments made by the writer<sup>3</sup> some years ago likewise tended to

<sup>1</sup> HEIDENHAIN: *Archiv für die ges. Physiol.*, 1875, x, p. 579.

<sup>2</sup> LINDBERGER: *Jahresbericht für Thierchemie*, 1883, p. 282.

<sup>3</sup> CHITTENDEN and CUMMINS: Influence of bile, bile salts, and bile acids on amylolytic and proteolytic action, *Amer. chem. journal*, 1885, vii, p. 50.

show that the presence of bile in a pancreatic extract containing combined salicylic acid may increase somewhat the rate of trypsin-proteolysis over that of the acid mixture alone. It was also shown at the same time that the addition of bile, even to the extent of 10 per cent, to neutral or alkaline pancreatic juice modifies only slightly the rate and extent of proteolysis; under some conditions inducing a slight stimulation and under other conditions a more marked inhibition of proteolysis. It was likewise observed that the deleterious action of combined hydrochloric acid upon trypsin-proteolysis was not overcome by the addition of bile. A few experiments reported by Martin and Williams<sup>1</sup> have also tended to indicate that bile and bile salts may stimulate somewhat the rate of pancreatic proteolysis.

A careful survey of the results, and of the conditions under which the results were obtained, recorded up to this time, led the writer to the conclusion that the addition of bile to a neutral or alkaline pancreatic juice causes but little change in its proteolytic action. Some slight stimulation may be produced, but there is no convincing proof that this is of constant occurrence or sufficient in degree to possess much physiological significance. We have been more inclined to the view that while the presence of bile in the intestine may be of primary importance for the assimilation of fats, its action upon trypsin-proteolysis is chiefly negative; *i. e.*, it neither retards nor stimulates proteolysis to any very great degree, under ordinary conditions. Recently, however, another paper<sup>2</sup> bearing on this subject has appeared which renders necessary a reconsideration of this question, for the results which the paper presents are so at variance with the above conclusions and so out of harmony with generally accepted views that some explanation of the apparent divergence must be sought. This is all the more necessary from the fact that comparatively few systematic quantitative experiments have been tried. We have therefore attempted a thorough study of the subject with a view to establishing firmly the nature and extent of the action which bile and its constituents exercise upon pancreatic proteolysis.

In the experiments reported by Rachford and Southgate emphasis is laid upon the fact that they were "planned for the purpose of throwing some light on the proteolytic action of pancreatic juice, under the conditions which normally exist in the duodenum." With this end in view pure pancreatic juice was obtained from rabbits,

<sup>1</sup> MARTIN and WILLIAMS: *Proceedings of the Royal Society*, 1890, xlviii, p. 160.

<sup>2</sup> RACHFORD and SOUTHGATE: *Medical record*, 1895, xlviii, p. 878.

through a pancreatic fistula, one rabbit yielding about 1 c.c. of the secretion in from four to six hours, this quantity sufficing for one experiment. As to the character of the bile employed there is no mention. We call attention to these facts because there is a manifest disposition on the part of these writers to accept the results of other workers in this field as conclusive for the pancreatic extracts, etc. employed, while their own divergent results are to be accepted as equally conclusive for the natural pancreatic juice. We are disinclined, however, to admit the correctness of this view. Pancreatic juice owes its proteolytic power to a specific enzyme. If the digestive power of this secretion is modified by the presence of bile through a specific action upon the enzyme it is clear that this influence will be exerted whether we are dealing with the natural secretion or with an extract of the gland. If, however, the influence exerted is an indirect one, affecting the enzyme only through changes of reaction, etc. it is equally manifest that this influence can be detected and measured to the best advantage when the environment is thoroughly known. We see, therefore, no particular advantage in making use of the natural secretion in a study of this kind, especially where the volume available is so small as to render the attainment of accurate quantitative results somewhat difficult. It is true theoretically that the addition of the fresh bile of an animal to the natural pancreatic juice of the same animal may constitute an ideal method for studying the influence of the former upon the activity of the latter; but when the exigencies of the case require that the digestive mixture be made by taking 5 drops of the natural pancreatic juice, adding 60 drops of water and 50 drops of a 4 per cent solution of bile,<sup>1</sup> we see very little reason for believing that the environment is thereby made to approximate any more closely to normal conditions than with the use of artificial extracts of the gland. The point involved is to our minds an important one, aside from the bearing it has upon the question before us. For if it is true that the action of a given agent upon a specific enzyme or upon the specific power of the enzyme, is necessarily different when added to the natural secretion in which the enzyme is contained from that which results when the same agent is added to an extract of the enzyme, then much of our knowledge regarding the conditions modifying and regulating the action of the digestive enzymes is of questionable value.

Rachford and Southgate seemingly incline to the view that the con-

<sup>1</sup> RACHFORD and SOUTHGATE : *loc. cit.*

ditions under which their experiments were carried out approach closely those normally existent in the duodenum. Granting that this may be so, one is still inclined after careful scrutiny of the conditions prevailing in their experiments to wonder what the *actual* conditions really were. Nowhere is there any mention made of the reaction of the fluids employed, nor of the reaction of the resultant mixture when bile, hydrochloric acid, and pancreatic juice were combined. To state merely that a given digestive mixture was prepared by adding 8 drops of pure pancreatic juice, 30 drops of 0.1 per cent hydrochloric acid, and 50 drops of a 4 per cent solution of bile leaves one in great doubt as to whether the mixture so manufactured was acid, alkaline, or neutral, and if acid whether it contained free or only combined acid. These are obviously very important elements to know if definite conclusions are to be drawn in explanation of the results. Data of this sort, however, are wholly wanting in the paper in question, hence we are forced simply to take the results and guess at the actual conditions under which they were obtained. Further, so-called pure pancreatic juice, like the other digestive secretions, is subject to constant modification by the character and extent of the stimulation which calls it forth, while the character and condition of the semi-digested food passing from the stomach into the duodenum, together with the amount of free and combined acid, must necessarily lead to variable conditions in the duodenum. Add to this the well known variations in the rate of flow and composition of the bile, and we may well ask what are the conditions which normally prevail in the duodenum? Obviously, we cannot make a definite answer within very close limits, for the conditions are bound to be more or less variable. What we have to ascertain, therefore, is the influence of bile and its constituents upon the proteolytic action of the pancreatic juice or its specific enzyme under the various conditions which are liable to exist in the upper part of the small intestine. This in our judgment can be studied to the best advantage by the use of artificial pancreatic juice, or extracts of the active gland, where the quantity obtainable will be sufficient to admit of comparative experiments under definite conditions. Moreover, the artificial pancreatic juice will not be widely different in the character of the proteid matter present from the natural secretion employed by Rachford and Southgate. Thus, these investigators state that from four to six hours were required for about one cubic centimetre of the pancreatic juice to flow from the fistula. Plainly, during this interval the active proteolytic enzyme present would transform the natural

proteids of the juice into peptone, amido-acids, etc., as completely as the transformation would be accomplished in the extracts themselves. Hence in this respect, at least, both fluids must differ from the natural secretion normally poured into the intestine.

#### I. METHODS EMPLOYED.

The pancreatic extracts employed in our experiments were prepared from two kinds of glands and by two distinct methods. 1. Using Kühne's well known method,<sup>1</sup> the fresh pancreatic glands of oxen were freed from fat and thoroughly dehydrated by long soaking in a large volume of strong alcohol and lastly in ether. To prepare the extract, 20 grams of the dry tissue were warmed at 40° C. for 24 hours with 200 c.c. of 0.1 per cent salicylic acid, the solution strained off, filtered through paper, made exactly neutral with sodium carbonate, and diluted with water to 1 litre. The sodium salicylate formed on neutralization will serve to prevent putrefaction for short periods, while the addition of a little thymol will preserve the fluid indefinitely. 2. Using Roberts's method,<sup>2</sup> pancreatic glands from pigs, freed from fat, were ground up with broken glass and soaked in four times their weight of 20 per cent alcohol for 4-5 days with frequent agitation. The extract was then filtered through paper, yielding a clear, slightly yellow fluid of strong proteolytic power.

The proteid material used in measuring the relative proteolytic power of the mixtures was purified blood fibrin, prepared by soaking carefully selected, well-washed fibrin in cold and boiling water until all soluble matter was removed, after which the fibrin was thoroughly extracted with cold and boiling alcohol and lastly with ether. The friable mass was then ground to a coarse powder and the latter passed through a series of sieves so as to bring together particles of the same size. It was then dried at 110° C. until of constant weight, and preserved for use. In some few experiments coagulated egg-albumin was made use of, in which case the actual content of dry proteid was determined by drying a given weight of the coagulum at 110° C. and then determining the ash by ignition.

The character of the bile employed is specified in each experiment. The secretion was always obtained as fresh as possible, and when from dogs or cats it was usually obtained through a temporary biliary fistula.

In arranging the experiments each digestive mixture in a series was

<sup>1</sup> KÜHNE: Untersuchungen aus d. physiol. Institute, Heidelberg, 1878, I., p. 223.

<sup>2</sup> ROBERTS: On the digestive ferments, etc., The Lumleian Lectures, London, 1880.

made to contain the same volume of pancreatic juice for each gram of dried fibrin — usually 10 or 20 c.c., according to the strength of the juice — with a total volume of 50 c.c.; water, bile, bile salts, acid, alkali, etc., being added in the proportions necessary to give the percentages specified. All the mixtures of a series were placed in the same water-bath at 40° C., all stirred equally, and when digestive action was sufficiently advanced the undigested residues were collected on weighed filters, previously dried at 110° C. in glass-stoppered weighing bottles, and washed thoroughly with hot water, and lastly with alcohol and ether. On drying the papers with their contents at 110° C. until of constant weight the proportion of undigested matter was readily ascertained, from which the relative proteolytic action of the several mixtures was easily calculated. It is hardly necessary to add that all the mixtures of a given series were kept at 40° C. for the same length of time, usually 2–4 hours, it being our aim to have the proteid material in the control mixture digested to the extent of 40–60 per cent.

## II. INFLUENCE OF FRESH BILE ON THE PROTEOLYTIC ACTION OF THE PANCREATIC ENZYME IN NEUTRAL SOLUTION.

The following quantitative results show the extent and character of the influence exerted by bile from various sources upon neutral solutions of trypsin.

### Experiment 1. Pig's bile. Neutral extract of pig's pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.4382 gram	56.18 per cent	100.0
0	0.4477	55.23	98.3
1.0	0.4891	51.09	90.9
3.0	0.4922	50.78	90.3
5.0	0.4616	53.84	95.8
10.0	0.4426	55.74	99.2
15.0	0.4204	57.96	103.1
20.0	0.4052	59.48	105.8
25.0	0.4206	57.94	103.1

### Experiment 2. Pig's bile. Neutral extract of pig's pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.4218 gram	57.82 per cent	100.0
0	0.4330	56.70	98.0
1.0	0.4690	53.10	91.8
3.0	0.4705	52.95	91.5
5.0	0.4668	53.32	92.2
10.0	0.4528	54.72	94.6
15.0	0.4172	58.28	100.8



**Experiment 3.** Pig's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3823 gram	61.77 per cent	100.0
0.25	0.4039	59.61	96.5
0.5	0.4175	58.25	94.3
1.0	0.4948	50.52	81.7
2.5	0.4815	51.85	83.9
5.0	0.5100	49.00	79.3

**Experiment 4.** Ox bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.2873 gram	71.27 per cent	100.0
1.0	0.3203	67.97	95.3
2.0	0.3203	67.97	95.3
3.0	0.3255	67.45	94.6
5.0	0.3306	66.94	93.9
10.0	0.3323	66.77	93.6
15.0	0.3019	69.81	97.9

**Experiment 5.** Ox bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.6008 gram	39.92 per cent	100.0
1.0	0.5880	41.20	103.2
2.0	0.6049	39.51	98.9
3.0	0.6157	38.43	96.2
4.0	0.6287	37.13	93.0
5.0	0.6228	37.72	94.4
10.0	0.6179	38.21	95.7

**Experiment 6.** Dog's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.4070 gram	59.30 per cent	100.0
1.0	0.4165	58.35	98.3
2.0	0.4175	58.25	98.2
3.0	0.4348	56.52	95.3

**Experiment 7.** Dog's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3353 gram	66.47 per cent	100.0
0.25	0.3389	66.11	99.4
0.5	0.3295	67.05	100.8
1.0	0.3094	69.06	103.8
2.5	0.3579	64.21	96.6
5.0	0.3760	62.40	93.8

**Experiment 8.** Dog's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.4535 gram	54.65 per cent	100.0
0.25	0.4602	53.98	98.7
0.5	0.4541	54.59	99.8
1.0	0.4461	55.39	101.3
2.5	0.4370	56.30	103.0
5.0	0.4045	59.55	108.9
10.0	0.4188	58.12	106.3
15.0	0.4155	58.45	106.9

**Experiment 9.** Cat's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.4189 gram	58.11 per cent	100.0
0	0.4226	57.74	99.3
2.0	0.4453	55.47	95.4
7.0	0.4124	58.76	101.1

**Experiment 10.** Sheep's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.5161 gram	48.39 per cent	100.0
0.25	0.5098	49.02	101.3
0.5	0.5187	48.13	99.4
1.0	0.5271	47.29	97.7
2.5	0.5383	46.17	95.4
5.0	0.5018	49.82	102.9

**Experiment 11.** Sheep's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.2409 gram	75.91 per cent	100.0
0.25	0.2301	76.99	101.4
0.5	0.2709	72.91	96.0
1.0	0.2645	73.55	96.8
2.5	0.3011	69.89	92.0
5.0	0.3370	66.30	87.3
10.0	0.3142	68.58	90.3

**Experiment 12.** Sheep's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.4826 gram	51.74 per cent	100.0
0.25	0.4754	52.46	101.3
0.5	0.4717	52.83	102.1
1.0	0.4931	50.69	98.0
2.5	0.4685	53.15	102.7
5.0	0.4689	53.11	102.6
10.0	0.4383	56.17	108.5

**Experiment 13.** Sheep's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3425 gram	65.75 per cent	100.0
0.5	0.3356	66.44	101.4
1.0	0.3149	68.51	104.2
2.5	0.3258	67.42	102.5
5.0	0.3427	65.73	99.9
10.0	0.3287	67.13	102.0

**Experiment 14.** Sheep's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.5791 gram	42.09 per cent	100.0
0.25	0.5705	42.95	102.0
0.5	0.5637	43.63	103.6
1.0	0.5635	43.64	103.6
2.5	0.5772	42.28	100.4
10.0	0.5414	45.86	108.9

**Experiment 15.** Sheep's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3507 gram	64.93 per cent	100.0
1.0	0.3499	65.01	100.9
2.5	0.3492	65.08	101.0
5.0	0.3569	64.31	99.0
10.0	0.3132	68.68	105.7

**Experiment 16.** Sheep's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3305 gram	66.95 per cent	100.0
0.25	0.3282	67.18	100.3
0.5	0.3243	67.57	101.4
1.0	0.3344	66.56	99.4
2.5	0.3446	65.54	97.8
5.0	0.3398	66.02	98.6
10.0	0.3076	69.24	103.4

**Experiment 17.** Human bile.<sup>1</sup> Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3042 gram	69.58 per cent	100.0
1.0	0.2980	70.20	100.8
2.0	0.3175	68.25	98.0
3.0	0.3542	64.58	92.8
5.0	0.3652	63.48	91.2
10.0	0.3833	61.67	88.6
20.0	0.4335	56.65	81.4
40.0	0.4305	56.95	81.8

<sup>1</sup> Containing 17.96 per cent of solid matter.

In looking through these various experiments it is manifest that the addition of fresh bile to a neutral pancreatic extract does not give rise to any very great degree of stimulation, *i. e.*, the proteid-digesting power of the enzyme is not markedly increased. Calling the digestive power of the control mixture without bile 100, it is rare to find the digestive power of the enzyme raised above 108 by addition of bile. Increased proteolysis, however, is certainly induced many times by the addition of bile, and it is somewhat noticeable that this increase is obtained more frequently in the presence of large percentages — 10–25 per cent — than in the presence of smaller amounts. Still, in no one of our experiments do we find a confirmation of the results reported by Rachford and Southgate, who found on an average “that the proteolytic action of pancreatic juice on neutral fibrin was increased one-fourth by the addition of bile.” Further, it is noticeable in our experiments that in full 50 per cent of the results inhibition of proteolysis is produced, although here, likewise, the retarding effect is not very marked. Rarely does the relative proteolytic action fall below 90. Our results, therefore, seemingly justify the statement that the addition of bile to a neutral solution of the pancreatic enzyme, even to the extent of 25 per cent, does not materially modify its proteolytic power; stimulation or inhibition may result, but not, under ordinary circumstances, to any very great degree. Still, the question arises at once why we have both stimulation and retardation. Obviously, some reason must exist for this apparent discrepancy in the results. First, however, we must call attention to the extent to which our analytical data can be trusted. Many times, to be sure, duplicate results agree very closely, but experience has taught us that, under the conditions of our experiments, the limit of error is about 1 per cent. This means that where 50–60 per cent of proteid matter is digested, relative proteolytic action may vary two points without having any special significance (see Experiments 1, 2, 9, 18, 20, and 30). It is plain, however, on carefully scrutinizing the preceding data, keeping in mind what has just been stated, that in some experiments bile manifestly tends to produce slight inhibition of proteolysis, while in other experiments, apparently under the same conditions, increased proteolysis results. It is further manifest that this difference in action is to be connected mainly with the character of the bile employed. Thus, it is to be noticed that increased proteolysis is much more common with sheep’s and dog’s bile than with ox or pig’s bile. Such differences in action might indeed be expected

when it is remembered how radically bile from different species of animals differs in composition. Still with sheep's bile stimulation of proteolysis is not constant, neither is inhibition always characteristic of pig's bile; but this is not strange when we recall how the bile from a given animal may vary in composition with changes in physiological conditions. Further, as Experiments 1 and 2 indicate, a given sample of bile may when present in one proportion retard proteolysis, while a larger percentage will accelerate digestion. This suggests the possible presence in bile of two opposing factors, one tending to accelerate, the other tending to retard proteolysis. Clearly such action as is produced cannot be due solely to the characteristic bile-salts which are contained in such abundance in the bile.

Bile is usually considered an alkaline-reacting fluid. Thus, Neumeister<sup>1</sup> states that "the reaction of bile is alkaline; it contains about 0.2 per cent sodium carbonate and about the same amount of alkaline-reacting sodium phosphate." It is true that fresh bile usually reacts alkaline to red litmus paper, but we have been unable to find any statements in the literature justifying the assumption that sodium carbonate is present. Indeed, some comparatively recent observations made by Jolles<sup>2</sup> show that the bile of oxen, dogs, and pigs, as well as human bile, reacts acid to phenolphthaleïn. This obviously does not imply the presence of free acid, although some free fatty acids may be present, such as stearic, palmitic, and oleic acids.<sup>3</sup> Jolles, indeed, concludes from his experiments that the fresh bile of man and the above-mentioned animals is not an alkaline or neutral fluid, but possesses a weak acid reaction. He finds, for example, on titrating ox-bile with a decinormal solution of potassium hydroxide, using phenolphthaleïn as an indicator, that on an average 1 gram of bile requires 0.546 milligram KOH to neutralize the free acids or acid-salts present. It is to be noted, likewise, that the acidity varies with different samples of bile, the extremes in ten experiments being 0.483 and 0.633. In pig's bile the average acidity was somewhat higher, 0.86 milligram KOH being required to neutralize the acid salts of 1 gram of bile. Further, the variations in acidity were much greater, the extremes in eight observations being 0.56 and 1.56. In dog's bile, on the other hand, the acidity (in one experiment) was only 0.42. Human bile, however, was much more

<sup>1</sup> NEUMEISTER: *Lehrbuch d. physiol. Chemie*, zweite Auflage, 1897, p. 195.

<sup>2</sup> JOLLES, A.: *Archiv f. d. ges. Physiol.*, 1894, lxxvii, p. 1.

<sup>3</sup> LASSAR-COHN: *Zeitschr. f. physiol. Chemie*, 1893, xvii, p. 607.

strongly acid, 1 gram of bile requiring on an average 2.36 milligrams of KOH to neutralize the acid salts. These observations, which have an important bearing upon the subject under consideration, we are able to confirm in a general way through a large number of determinations made in this laboratory<sup>1</sup> upon various kinds of bile. Only in rabbit's bile was there a failure to detect a measurable amount of acidity. Cat's bile, however, showed an acidity equal to only 0.23. Now it is obvious from these statements that bile (excepting possibly rabbit's bile) cannot contain any alkali as strong as sodium carbonate. Such alkaline reaction as bile yields with red litmus, lacmoid, etc., must be due to the presence of such salts as  $\text{Na}_2\text{HPO}_4$ ,  $\text{NaH}_2\text{PO}_4$ , etc. We may measure the amount of alkalinity in bile, using lacmoid as an indicator, by titration with a solution of decinormal hydrochloric acid. Using this method, Mr. Brown found on an average that pig's bile having an acidity equal to 0.50 milligram NaOH per gram had an alkalinity equal to 1.05 milligram HCl per gram. Sheep's bile with an average acidity of 0.45 possessed an alkalinity of 0.91. Ox bile with an average acidity of 0.43 showed an average alkalinity of 1.50, while rabbit's bile flowing directly from the liver through a fistula and not coming in contact with the gall bladder had an alkalinity of 2.9 without any measurable acidity. These statements would seem to show that the alkalinity as indicated by lacmoid is considerably greater than the acidity as indicated by phenolphthalein, but this is not always the case, for in some of our experiments, to be quoted shortly, it will be observed that the acidity frequently predominates. We would call special attention, however, to the observation made with rabbit's bile, for if it is true that the latter fluid invariably has a strong alkalinity, the addition of such a bile to neutral pancreatic juice would obviously accelerate proteolysis. If Rachford and Southgate used by chance the bile of the rabbit in their experiments, it might help explain the great acceleration of digestion noticed by them on addition of bile to "neutral fibrin." The point, however, which we wish to emphasize is that the bile of most animals under ordinary conditions is not a strongly alkaline fluid, that it contains no such alkali as sodium carbonate, but on the other hand is possessed of a weak acidity due to the presence of certain acid salts, such as the phosphates of the alkalies, together with possible weak organic acids or other organic compounds. The fact that the acid-reacting bile fails to produce any effect on blue lacmoid is convincing

<sup>1</sup> By Ernest W. Brown, Ph. B.

proof that the fluid does not contain free organic acids of any strength. On the other hand, fresh bile does possess a certain degree of alkalinity to be detected by litmus and lacmoid, but due mainly at least to the presence of salts or compounds which are either acid to phenolphthaleïn, or which exist side by side with such compounds. Lastly, we would emphasize the fact already brought out, that the so-called acidity of the bile, and likewise the so-called alkalinity, have different values in different species of animals, and may likewise vary in the same species under different conditions of diet, etc.

The bearing of these facts upon the problem before us is sufficiently manifest. The addition of bile to a neutral pancreatic fluid (neutral to litmus) must plainly introduce a change in the reaction, as measured by either litmus, lacmoid, or phenolphthaleïn. Further, owing to the variations in the acidity and alkalinity, already referred to, it is clear that different samples of bile will produce different results, and when it is remembered how sensitive the proteolytic enzyme trypsin is toward changes of reaction, it is obvious that this feature cannot be overlooked in considering the influence of bile upon pancreatic proteolysis.

Let us consider now the character of the bile used in some of the preceding experiments on proteolysis.

		<i>Degree of Acidity.<sup>1</sup></i>	<i>Degree of Alkalinity.<sup>2</sup></i>
Experiment 2	Pig's bile . . . .	0.50	1.17
" 3	" . . . .	0.60	...
" 7	Dog's bile . . . .	0.90	0.54
" 8	" . . . .	0.76	0.40
" 12	Sheep's bile . . . .	0.50	0.37
" 13	" . . . .	0.40	0.91
" 14	" . . . .	0.60	0.73

These data show us at once that in introducing say 10 per cent of bile into the digestive mixtures, variations in reaction must necessarily ensue. Contrast, for example, the bile used in Experiments 2 and 7, also in 12 and 13. Here we have marked differences in the ratio of acid salts to alkaline salts, but without any appreciable difference in the relative proteolytic action of the mixtures. Everything else being equal we should expect to find increased proteolysis most marked in those mixtures where the bile introduced showed a predominance of alkaline salts, but no such conclusion can be drawn from the results. On the

<sup>1</sup> Expressed in milligrams NaOH required to neutralize one gram bile, phenolphthaleïn as indicator.

<sup>2</sup> Expressed in milligrams HCl required to neutralize one gram bile, lacmoid as indicator.

contrary, it is plain that such slight influence as bile exerts on the proteolytic action of *neutral* pancreatic juice is not connected primarily with change of reaction, but must be attributed to some other cause. These points, however, will be referred to again in another connection.

### III. INFLUENCE OF FRESH BILE ON THE PROTEOLYTIC ACTION OF THE PANCREATIC ENZYME IN ALKALINE SOLUTION.

The experiments embraced under this head were conducted in the same manner as those previously described, except that to each digestive mixture was added 0.125 gram of sodium carbonate. Hence, each mixture contained 0.25 per cent sodium carbonate, unless modified by the bile added. Following are the results obtained: —

#### Experiment 18. Pig's bile. Alkaline extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3721 gram	62.79 per cent	100.0
0	0.3790	62.10	98.9
1.0	0.4440	55.60	88.5
1.0	0.4350	56.50	89.9
2.0	0.4490	55.10	87.7
2.0	0.4699	53.01	84.4
5.0	0.4318	56.82	90.4
5.0	0.4230	57.70	91.8
10.0	0.4255	57.45	91.4
10.0	0.4198	58.02	92.4

#### Experiment 19. Pig's bile. Alkaline extract of pig's pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3769 gram	62.31 per cent	100.0
1.0	0.3752	62.48	100.2
10.0	0.3997	60.03	96.3
15.0	0.4037	59.63	95.6
25.0	0.4349	56.51	90.6
50.0	0.5048	49.52	79.4

#### Experiment 20. Pig's bile. Alkaline extract of pig's pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action</i>
0	0.1535 gram	84.65 per cent	100.0
0	0.1498	85.02	100.4
3.0	0.2080	79.20	93.5
5.0	0.2177	78.23	92.4
10.0	0.2462	75.38	89.0
15.0	0.2400	76.00	89.7
20.0	0.2603	73.97	87.3
25.0	0.2428	75.72	89.4
30.0	0.2560	74.40	87.8
40.0	0.2743	72.57	85.7



**Experiment 21.** Pig's bile. Alkaline extract of ox pancreas.<sup>1</sup>

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Albumin digested.</i>	<i>Relative proteolytic action.</i>
0	0.0891 gram	88.41 per cent	100.0
0	0.0832	89.20	100.8
2.0	0.1320	82.80	93.6
5.0	0.0765	90.07	101.8
15.0	0.0257	96.66	109.3
20.0	0.0262	96.47	109.1

**Experiment 22.** Ox bile. Alkaline extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.2482 gram	75.18 per cent	100.0
1.0	0.2565	74.35	98.8
2.0	0.2771	72.29	96.1
3.0	0.2929	70.71	94.0
4.0	0.2860	71.40	94.9
5.0	0.2862	71.38	94.9
10.0	0.3036	69.64	92.6
15.0	0.2827	71.73	95.4

**Experiment 23.** Calf's bile. Alkaline extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.2996 gram	70.04 per cent	100.0
1.0	0.3190	68.10	97.2
2.0	0.3409	65.91	94.1
3.0	0.3348	66.52	94.9
4.0	0.3314	66.86	95.4
5.0	0.3113	68.87	98.3
10.0	0.2662	73.38	104.6
15.0	0.2750	72.50	103.5

**Experiment 24.** Human bile.<sup>2</sup> Alkaline extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.2960 gram	70.40 per cent	100.0
0.5	0.3080	69.20	98.2
1.0	0.3091	69.09	98.1
2.0	0.3085	69.15	98.2
3.0	0.3074	69.26	98.3
5.0	0.3267	67.33	95.6
10.0	0.3360	66.40	94.3
20.0	0.3980	60.20	85.5
40.0	0.4904	50.96	72.3

<sup>1</sup> In this experiment, thoroughly washed coagulated egg-albumin was employed instead of blood fibrin. Each mixture contained originally 3 grams of the coagulum = 0.7707 gram of dry proteid.

<sup>2</sup> Containing 13.3 per cent of solid matter.

**Experiment 25.** Dog's bile. Alkaline extract of pig's pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.1598 gram	84.02 per cent	100.0
0	0.1779	82.21	97.8
0.5	0.1855	81.45	96.9
1.0	0.2237	77.63	92.3
5.0	0.2041	79.59	94.7
10.0	0.2433	75.67	90.0
15.0	0.2481	75.19	89.4

**Experiment 26.** Dog's bile. Alkaline extract of pig's pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.1884 gram	81.16 per cent	100.0
2.0	0.1513	84.87	104.5
4.0	0.1148	88.52	109.0
8.0	0.1470	85.30	105.1
15.0	0.1894	81.06	99.8

**Experiment 27.** Dog's bile. Alkaline extract of pig's pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3913 gram	60.87 per cent	100.0
5.0	0.3593	64.07	105.2
10.0	0.3622	63.78	104.6

**Experiment 28.** Dog's bile. Alkaline extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.5374 gram	46.26 per cent	100.0
20.0	0.5267	47.33	102.3

**Experiment 29.** Sheep's bile. Alkaline extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3935 gram	60.65 per cent	100.0
0.25	0.3880	61.20	100.9
0.5	0.4079	59.21	97.6
2.5	0.4236	57.64	95.3
5.0	0.4135	58.65	96.7
10.0	0.4002	59.98	98.8

**Experiment 30.** Sheep's bile. Alkaline extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3284 gram	67.16 per cent	100.0
0	0.3330	66.70	99.3
0.25	0.3293	67.07	99.8
0.5	0.3328	66.72	99.3
1.0	0.3151	68.49	101.9
2.5	0.2937	70.63	105.1
5.0	0.2913	70.87	105.5
10.0	0.2635	73.65	109.6
15.0	0.2419	75.81	112.8

On looking through these results and comparing them with those of the preceding series, it is apparent that the addition of bile to an *alkaline* pancreatic juice is liable to produce a greater relative retardation of proteolysis than the addition of the same amount to a *neutral* pancreatic fluid. An alkaline pancreatic juice, however, containing even 40 per cent of bile will digest as much proteid in a given time as the same pancreatic fluid neutralized but without the addition of bile. If the control mixtures of the two series are compared, it will be observed that the alkaline fluids dissolve on an average about 10–15 per cent more proteid than the neutral fluids. This is merely an illustration of the well-known fact that a weak alkaline fluid is much more favorable for pancreatic proteolysis than a neutral fluid. We are inclined to attribute such retardation of proteolysis as bile induces when added to an alkaline pancreatic fluid in great part to the reduction of alkalinity liable to occur. We say liable because this will depend primarily upon the relative proportion of acid and alkaline salts in the bile. When the latter contains a relatively large proportion of acid salts, as indicated by phenolphthaleïn, the addition of large percentages of such a bile will rapidly diminish the amount of sodium carbonate present, since the latter will be more or less used up in transforming the acid compounds into neutral ones. This is well illustrated in Experiment 20, wherein actual examination showed that at the conclusion of the digestion all the mixtures containing more than 15 per cent of bile had lost entirely their alkaline reaction toward litmus. In other words, the acid-reacting compounds contained in 10 grams of this bile were sufficient to neutralize the 0.125 gram of sodium carbonate originally present in the mixture. In harmony with this fact it is to be noted throughout these latter experiments that retardation is most marked in the presence of those biles which have been shown to have as a rule the highest acidity, viz., pig's bile and human bile. With dog's bile, on the other hand, in which acidity is usually very slight, no retardation whatever was observed; all three experiments gave evidence of some slight stimulation of proteolysis. The occasional stimulation noticed with sheep's bile and pig's bile we attribute in part to the lower acidity of the samples used. Leaving these points out of consideration, however, and turning our attention to the collected data, it is plain that the addition of even 40 or 50 per cent of bile to an alkaline pancreatic fluid does not greatly retard the proteolytic action of the enzyme (see Experiments 19, 21, and 24), certainly no more than would result from neutralization of the alka-

linity. By this we do not mean that the specific bile salts are without influence on pancreatic proteolysis, but merely that the changes in reaction resulting from addition of normal bile are in themselves sufficient to account for the retardation noticed. Similarly, such stimulation of proteolysis as results may be due as much to a more favorable change in the reaction of the mixture as to any other cause.

#### IV. INFLUENCE OF BILE SALTS ON THE PROTEOLYTIC ACTION OF THE PANCREATIC ENZYME IN NEUTRAL AND ALKALINE SOLUTION.

In considering the action of the salts of the bile acids on pancreatic proteolysis it is to be remembered that in ox bile both glycocholate and taurocholate of sodium are present, although the proportion of the two acids is subject to considerable variation. In most cases, when ox bile is acidified, the proportion of taurocholic acid present is sufficient to hold the less soluble glycocholic acid in solution, but in some cases the latter predominates to such an extent that it will crystallize out under the above conditions. Thus Marshall<sup>1</sup> found as the result of an examination of 543 samples of bile from as many oxen that a separation of glycocholic acid could be obtained in only 121 cases, *i. e.*, in 22.2 per cent. This fact is worthy of note in the present connection as illustrating not only the variable composition of bile from a given species, but also as an indication of the variability of composition to be expected in the preparation of "crystallized bile."<sup>2</sup> Further, in pig's bile we have to do mainly with the sodium salts of two special forms of glycocholic acid known as  $\alpha$ - and  $\beta$ -hyo-glycocholic acid.<sup>3</sup> In the preparation of crystallized bile, *i. e.*, the sodium salts of the above acids, the ordinary methods of procedure were followed, and need not be detailed here.

Following are the results obtained in studying the influence of these preparations on pancreatic proteolysis under the conditions specified.

<sup>1</sup> MARSHALL, J. : *Zeitschr. f. physiol. Chemie*, 1887, ii, p. 233.

<sup>2</sup> For the variation in the proportion of glycocholate and taurocholate present in human bile see the results reported by Hammarsten: *Zur Kenntniss der Lebergalle des Menschen*. Mitgetheilt der königl. Gesellsch. d. Wissenschaften zu Upsala, 15 Juni, 1893. Separatabzug.

<sup>3</sup> JOLIN, S. : *Zeitschr. f. physiol. Chemie*, 1888, xii, p. 512, and xiii, p. 205.

**Experiment 31.** Crystallized ox-bile salts. Neutral extract of ox pancreas.

<i>Per cent of Bile salts.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.2933 gram	70.67 per cent	100.0
0	0.2707	72.93	103.1
1.0	0.2819	71.81	101.6
2.0	0.2579	74.21	105.0
3.0	0.3144	68.56	97.0
5.0	0.3790	62.10	87.8

**Experiment 32.** Crystallized ox-bile salts. Neutral extract of ox pancreas.

<i>Per cent of Bile salts.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.3866 gram	61.34 per cent	100.0
0.25	0.4481	55.19	89.9
1.00	0.4675	53.25	86.8
2.00	0.4651	53.49	87.2
3.00	0.4528	54.72	89.2
5.00	0.4360	56.40	91.9

**Experiment 33.** Crystallized ox-bile salts. Alkaline<sup>1</sup> extract of ox pancreas.

<i>Per cent of Bile salts.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.4940 gram	50.60 per cent	100.0
0	0.4970	50.30	99.4
1.0	0.4729	52.71	104.1
2.0	0.4465	55.35	109.3
3.0	0.4722	52.78	104.3

**Experiment 34.** Pure crystallized sodium glycocholate. Alkaline extract of ox pancreas.

<i>Per cent of Glycocholate.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.5374 gram	46.20 per cent	100.0
0	0.5277	47.23	102.2
1.0	0.5459	45.41	98.3
2.0	0.5573	44.27	95.8
3.0	0.5987	40.13	86.8

**Experiment 35.** Bile salts from pig's bile. Neutral extract of pig's pancreas.

<i>Per cent of Bile salts.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0.	0.5781 gram	42.19 per cent	100.0
0.5	0.6197	38.03	90.1
1.0	0.6427	35.73	84.6
2.0	0.6894	31.06	73.6
3.0	0.7105	28.95	68.6

**Experiment 36.** Bile salts from pig's bile. Alkaline extract of pig's pancreas.

<i>Per cent of Bile salts.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.4935 gram	50.65 per cent	100.0
0.5	0.4989	50.11	98.9
1.0	0.4750	52.50	103.6
2.0	0.4709	52.91	104.4
3.0	0.5383	46.17	91.1

<sup>1</sup> Each mixture containing 0.25 per cent sodium carbonate.

The contrast between the results obtained in the two preceding experiments led to our testing the reaction of the bile salts prepared from pig's bile, and it was found that they were quite strongly acid to litmus. It is thus evident that the marked inhibition of proteolysis observed in Experiment 35 was due, in part at least, to the increasing acidity of the mixtures. In Experiment 36, on the other hand, the 0.125 gram of sodium carbonate present overcame this acidity except in the mixture containing 3.0 per cent of the bile salts.

**Experiment 37.** Bile salts from pig's bile. Alkaline extract of pig's pancreas. These bile salts were exceedingly acid, hence 0.25 gram  $\text{Na}_2\text{CO}_3$  was added to each mixture, thus making the amount of this salt 0.5 per cent in the control. In the presence of 3.0 per cent of the bile salts, however, the alkalinity was reduced to one-fifth.

<i>Per cent of Bile salts.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.1752 gram	82.48 per cent	100.0
0.5	0.2045	79.55	96.4
1.0	0.2142	78.58	95.2
2.0	0.2282	77.18	93.5
3.0	0.3281	67.19	81.4

**Experiment 38.** Bile salts from pig's bile, made neutral before addition. Alkaline extract of pig's pancreas. Each mixture containing 0.25 per cent  $\text{Na}_2\text{CO}_3$ , as usual.

<i>Per cent of Bile salts.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.1846 gram	81.54 per cent	100.0
0.5	0.2249	77.51	95.0
1.0	0.2207	77.93	95.5
2.0	0.2222	77.78	95.3
3.0	0.2324	76.76	94.1
5.0	0.2543	74.57	91.4

**Experiment 39.** Bile salts from pig's bile, made neutral before addition. Extract of ox pancreas.

<i>Character of the Fluid.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral + 0 Bile salts . . . .	0.5499 gram	45.01 per cent	100.0
" + 1.0% " . . . .	0.6467	35.33	78.4
" + 3.0% " . . . .	0.6966	30.34	67.4
" + 5.0% " . . . .	0.7701	22.99	51.0
0.25 % $\text{Na}_2\text{CO}_3$ + 0 Bile salts . .	0.2748	72.52	161.1
" " + 1.0% " . .	0.3992	60.08	133.4
" " + 3.0% " . .	0.5238	47.62	105.7
" " + 5.0% " . .	0.6750	32.50	72.2

A critical examination of the foregoing results shows us first that the bile salts from ox bile have no very great influence in either direction upon pancreatic proteolysis. In two experiments (31 and

33) there is some evidence of acceleration, while in one experiment retardation is more noticeable. With pure sodium glycocholate (3 per cent) relative proteolytic action is reduced from 100 to 87. In Experiment 47, to be quoted later, stimulation of proteolysis is quite marked. In considering these results, however, in their bearing on the influence of fresh bile, it is to be remembered that 3-5 per cent of these bile salts are equivalent to the addition of 40-50 per cent of the original bile. With regard to the apparent difference in action of the several samples of ox bile salts we are inclined to attribute this to variations in the proportion of glycocholate and taurocholate present. Pure glycocholate, other influences excluded, seems to have a greater inhibitory action than the mixed salts, and possibly the more pronounced retardation seemingly characteristic of the salts from pig's bile is due to the fact that the salts are mainly glycocholates. Still it is to be observed that the samples of salts from pig's bile vary considerably in the intensity of their action, and this independently of their acidity, for when the latter is neutralized the same retarding effect is still produced. Somewhat noticeable also is the difference in intensity of action of the neutralized bile salts (from pig's bile) when added to the pancreatic juice of the same species as contrasted with the result obtained when the salts are added to the pancreatic extract from another species (contrast Experiments 38 and 39). The results collectively certainly warrant the conclusion that the isolated bile salts taken by themselves do not exert any very marked stimulation of pancreatic proteolysis. They may, on the other hand, give rise to some retardation, — an effect which is seemingly more characteristic of the salts from pig's bile than of those common to ox bile. It is to be noted, however, that the salts from pig's bile were not so pure chemically as the crystallized salts separated from ox bile, but frequently showed an acid reaction.

The above somewhat unsatisfactory results have served to strengthen our conviction that such limited action as normal bile exerts on pancreatic proteolysis is the result mainly of influence on the reaction of the digestive mixture, and that many agencies other than the specific bile salts are concerned. No doubt, some of these are more or less antagonistic to each other. Thus, pig's bile, as has been frequently stated by many observers, is liable to be extremely viscid, but the viscosity is not always conspicuous; at times the bile is quite limpid. This viscosity is due, in great part at least, to a mucin or nucleal-

bumin, precipitable by alcohol, and we have found that when this substance is removed from the bile there is a noticeable difference in the influence of the fluid on proteolysis. We may cite the following experiment:

Fresh pig's bile, very viscid, having a specific gravity of 1036, an acidity of 0.58, and an alkalinity of 1.15, was treated with five volumes of strong alcohol, the precipitate filtered off, and washed with alcohol. The united filtrate and washings were then evaporated for the removal of the alcohol, and the fluid made up with distilled water to the original volume. The acidity was now 0.48, and the alkalinity 0.90. The action of a portion of the original fresh bile and of the bile freed from nuclealbumin, etc., on proteolysis was then tested.

**Experiment 40.** With fresh pig's bile. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.5777 gram	42.23 per cent	100.0
0.25	0.6088	39.12	92.6
0.50	0.6241	37.59	89.0
1.00	0.7000	30.00	71.0
2.50	0.7118	28.82	68.2
5.00	0.6496	35.04	82.9
10.00	0.7235	27.65	65.4

**Experiment 41.** With bile freed from nuclealbumin. Neutral extract of ox pancreas.

<i>Per cent of Bile.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0	0.6446 gram	35.54 per cent	100.0
0.25	0.6556	34.44	96.9
0.50	0.6651	33.49	94.2
1.00	0.7007	29.93	84.2
2.50	0.7351	26.49	74.5
5.00	0.7217	27.83	78.3
10.00	0.6598	34.02	95.7

It is noticeable from these two experiments that the removal of the nuclealbumin, with possibly some of the inorganic salts from pig's bile, diminishes in a general way the retarding effect of the latter on proteolysis. Somewhat noticeable also is the peculiar relationship in the rise and fall of proteolysis under the influence of different percentages of the two samples.

With ox bile an attempt was made to separate the fluid into three distinct fractions, using methods which would presumably cause little or no change in the nature or composition of the various constituents. For this purpose 440 c.c. of fresh ox bile, containing 12.4 per cent of solid matter, were evaporated to a very thick syrup on the water-bath and precipitated with absolute alcohol. The small precipitate which



resulted was filtered off, washed thoroughly with alcohol, and then dried over sulphuric acid. It weighed 2.27 grams. The alcoholic filtrate was treated with a large volume of ether, the precipitated bile salts filtered off, washed thoroughly with ether, and dried. The alcohol-ether filtrate was allowed to evaporate, and finally brought to complete dryness on the water-bath. The effect of these three fractions on pancreatic proteolysis was then determined in the usual manner. The bile salts and the residue from the alcohol-ether filtrate were readily soluble in water, but the alcoholic precipitate was not completely soluble. Following are the results obtained: —

**Experiment 42.** With a neutral extract of ox pancreas.

<i>Per cent of Bile Constituents.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0 . . . . .	0.4217 gram	57.83 per cent	100.0
0.5 Bile salts . . . . .	0.4533	54.67	94.5
1.0 " . . . . .	0.4452	55.48	95.9
1.5 " . . . . .	0.4415	55.85	96.5
0.25 Alcoholic p.p. . . . .	0.3862	61.38	106.1
0.5 " " . . . . .	0.3704	62.96	108.8
1.0 " " . . . . .	0.4059	59.41	102.7
0.5 Alcohol-ether filtrate . .	0.4147	58.53	101.2
1.0 " " . . . . .	0.4032	59.68	103.2
1.5 " " . . . . .	0.3759	62.41	107.9

**Experiment 43.** A duplicate of the preceding, except that an alkaline (0.25 per cent  $\text{Na}_2\text{CO}_3$ ) extract of ox pancreas was employed.

<i>Per cent of Bile Constituents.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
0 . . . . .	0.1886 gram	81.14 per cent	100.0
0.5 Bile salts . . . . .	0.2847	71.53	88.1
1.0 " . . . . .	0.2883	71.17	87.7
1.5 " . . . . .	0.2655	73.45	90.5
0.25 Alcoholic p.p. . . . .	0.2388	76.12	93.8
0.5 " " . . . . .	0.2345	76.55	94.3
1.0 " " . . . . .	0.2370	76.30	94.0
0.5 Alcohol-ether filtrate . .	0.2473	75.27	92.7
1.0 " " . . . . .	0.2929	70.71	87.1
1.5 " " . . . . .	0.2689	73.11	90.1

From the first of these two experiments, where a neutral pancreatic fluid was employed, it is seen that the fraction containing the bile salts produces a slight inhibition of proteolysis, while the other two fractions increase the proteolytic action of the enzyme. Also noticeable is the tendency of the material from the alcohol-ether filtrate to increase proteolysis in proportion to the amount added, while the favorable action of the alcoholic precipitate appears to diminish with

increase in the proportion used. That these peculiarities of action, however, are not due to any direct influence upon the proteolytic enzyme is evident from the fact that in the second experiment where the reaction of the digestive mixtures is alkaline these differences disappear. The bile salts still produce inhibition, but the other two fractions no longer give rise to increased proteolysis; on the contrary, they tend to check the rate of proteolysis. It is thus clearly evident that in bile there are present various elements capable under different conditions of producing divergent effects, in minor degree, upon pancreatic proteolysis — effects which may counterbalance each other to some extent.

#### V. INFLUENCE OF BILE AND BILE SALTS ON THE PROTEOLYTIC ACTION OF THE PANCREATIC ENZYME IN THE PRESENCE OF FREE AND COMBINED ACIDS.

It has been generally accepted, on the basis of what has seemed sufficient experimental evidence, that the proteolytic enzyme of the pancreas is practically inactive in the presence of free hydrochloric acid.<sup>1</sup> Even *free organic* acids inhibit almost completely the action of the enzyme. Further, the presence of combined acid, *i. e.*, combined with proteid matter, checks to a greater or less degree the activity of the digestive fluid. Rachford and Southgate, however, state: "in our experiment we have found that the proteolytic action of pancreatic juice on fibrin is quite as strong in a one-thirtieth per cent hydrochloric-acid solution as it is in a neutral solution. If there is any difference, in fact, it is in favor of the hydrochloric-acid solution." The statement is positive, but we cannot find in their paper any *conclusive* evidence as to the actual degree of acidity. Thus, to quote one of their experiments, 10 minims of pure pancreatic juice, 50 minims of water, and 30 minims of 0.1 per cent hydrochloric acid were mixed together; — but how much of this acid was used in neutralizing the alkalinity of the pancreatic juice, and how much was combined with the proteids of the secretion? Nowhere in their paper can we find any evidence of discrimination between free and combined acid, or any attempt to determine the actual percentage of either free or combined acid really present. The question is one of considerable physiological importance, and if it is true that the pancreatic juice acting in an

<sup>1</sup> CHITTENDEN and CUMMINS: Studies in physiol. chemistry. Yale Univer. 1885, vol. i, p. 135. This paper contains full references to other work in this direction.

acid solution will do more work than in a neutral solution, it should be clearly established. We have, therefore, first turned our attention to this point.

**Experiment 44.** With neutral extracts of ox pancreas and pig's pancreas (neutral to litmus).

10 c.c. of the extract of pig's pancreas required 4.95 c.c. 0.2 per cent HCl to combine with all the proteid matter present.<sup>1</sup>

10 c.c. of the extract of ox pancreas required 2.55 c.c. 0.2 per cent HCl to combine with the proteids.

In the digestions with pig's pancreatic fluid each mixture contained 10 c.c. of the extract, while with the pancreatic fluid from the ox pancreas 30 c.c. of extract were used in each case. Acid was then added to the mixtures as specified, the percentages being calculated on the total volume (50 c.c.) of the digestive mixtures. In no case was any free acid present.

<i>Ox pancreatic juice.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral . . . . .	0.3279 gram	67.211 per cent	100.0
Proteids combined with HCl—			
Quarter saturated (0.007% HCl) .	0.3782	62.18	92.5
Half “ (0.015% “ ) .	0.4995	50.05	74.4
Wholly “ (0.03% “ ) .	0.8710	12.90	19.1
<i>Pig's pancreatic juice.</i>			
Neutral . . . . .	0.0435	95.65	100.0
Proteids combined with HCl—			
One-sixth saturated (0.0016% HCl),	0.0469	95.31	99.6
Quarter “ (0.0025% “ ),	0.0550	94.50	98.7
Half “ (0.0049% “ ),	0.0826	91.74	95.9
Wholly “ (0.0099% “ ),	0.2239	77.61	81.1

**Experiment 45.** 10 c.c. of the extract of ox pancreas required 4.3 c.c. 0.2 per cent HCl to combine with the proteids.

10 c.c. of the extract of pig's pancreas required 4.6 c.c. 0.2 per cent HCl to combine with the proteids.

In the digestions, 10 c.c. of the extract of pig's pancreas, and 30 c.c. of the extract of ox pancreas were employed.

<i>Ox pancreatic juice.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral . . . . .	0.3896 gram	61.04 per cent	100.0
Proteids combined with acid—			
One-eighth saturated (0.006% HCl),	0.3951	60.49	99.0
One-sixth “ (0.008% “ ),	0.4798	52.02	85.2
One-fourth “ (0.012% “ ),	0.5030	49.70	81.4
One-half “ (0.025% “ ),	0.6509	34.91	57.1
Wholly “ (0.05% “ ),	0.9327	6.73	11.0

<sup>1</sup> Tropæolin OO in methyl alcohol was used as the indicator for free acid. Obviously, in conducting the titrations, deduction was made for the excess of acid required to bring out the color.

<i>Pig's pancreatic juice.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral . . . . .	0.1966 gram.	80.34 per cent.	100.0
Proteids combined with acid—			
One-sixth saturated (0.003% HCl),	0.2110	78.90	98.2
One-fourth “ (0.004% “ ),	0.2654	73.46	91.4
One-half “ (0.009% “ ),	0.3281	67.19	83.6
Wholly “ (0.018% “ ),	0.5564	44.36	55.2

**Experiment 46.** With extract of ox pancreas. 25 c.c. of the neutral extract required 19.7 c.c. 0.4 per cent *salicylic acid* to combine with the proteids present.

<i>Character of the fluid.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral . . . . .	0.4658 gram	53.42 per cent	100.0
“ . . . . .	0.4894	51.06	95.5
Proteids combined with acid—			
Completely saturated (0.157% acid),	0.9860	1.40	2.6
“ “ “	0.9874	1.26	2.3
Half “ (0.078% acid),	0.8443	15.57	29.1
“ “ “	0.8375	16.25	30.4

In considering the results of the three preceding experiments it is to be remembered that in no one of the digestive mixtures was there any *free* acid present, hence such effects as are produced come solely from the influence of the combined acid. Of the latter, even a few thousandths of one per cent suffice to exert an inhibitory influence on proteolysis, and with a sufficient amount of combined acid alone, even with a weak organic acid, proteolysis may be almost completely checked. We fail to see therefore how the addition of acid to a *neutral* pancreatic juice can increase the digestive power of the solution.

What now is the influence of bile and bile salts on pancreatic proteolysis in the presence of combined acid? The answer to this question is found in the results of the following experiments.

**Experiment 47.** With extract ox pancreas. Ox-bile salts.

<i>Character of the fluid.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral . . . . .	0.5291 gram	47.09 per cent	100.0
“ + 2% Bile salts . . . . .	0.5183	48.17	102.3
“ + 4% “ . . . . .	0.4818	51.82	110.0
Proteids combined with acid—			
Eighth saturated “ (0.005% HCl) .	0.5860	41.40	87.9
“ “ + 2% Bile salts .	0.5589	44.11	93.6
Quarter “ (0.01% HCl) .	0.6330	36.70	77.8
“ “ + 2% Bile salts .	0.6359	36.41	77.3
“ “ + 4% “ .	0.6230	37.70	80.0

**Experiment 48.** With extract of pig's pancreas. Bile salts from pig's bile.

<i>Character of the fluid.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral . . . . .	0.1755 gram	82.45 per cent	100.0
Proteids combined with HCl —			
Half saturated " (0.013% HCl) .	0.2337	76.63	92.9
" " " + 0.5% Bile salts,	0.4433	55.67	67.5
" " " + 1.0% " "	0.4039	59.61	72.2
" " " + 2.0% " "	0.4958	50.42	61.1
" " " + 3.0% " "	0.4992	50.08	60.7

**Experiment 49.** With extract of pig's pancreas. Bile salts from pig's bile. The salts somewhat acid in reaction. Salicylic acid used to combine with the proteids.

<i>Character of the fluid.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral . . . . .	0.3041 gram	69.59 per cent	100.0
" + 3% Bile salts . . . . .	0.5338	46.62	66.9
Proteids combined with acid —			
Wholly saturated " (0.064% ) .	0.5795	42.05	60.4
" " " + 3% Bile salts .	0.7608	23.92	34.3
Half " " (0.032% ) .	0.4296	57.04	81.9
" " " + 3% Bile salts .	0.6773	32.27	46.3
Quarter " " (0.016% ) .	0.3608	63.92	91.8
" " " + 3% Bile salts .	0.5591	44.09	63.3

**Experiment 50.** With extract of pig's pancreas. Bile salts from pig's bile made perfectly neutral. Salicylic acid used to combine with the proteids.

<i>Character of the fluid.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral . . . . .	0.2132 gram	78.68 per cent	100.0
Proteids combined with acid —			
Half saturated " (0.053% acid) .	0.6038	39.62	50.3
" " " + 0.5% Bile salts .	0.7659	23.41	29.7
" " " + 1.0% " "	0.8120	18.80	22.6
" " " + 2.0% " "	0.8319	16.81	21.3
" " " + 3.0% " "	0.8900	11.00	13.9

**Experiment 51.** With extract of ox pancreas. Fresh pig's bile. Salicylic acid used to combine with the proteids.

<i>Character of the fluid.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral . . . . .	0.4125 gram	58.75 per cent	100.0
Proteids combined with acid —			
Half saturated " (0.038% acid) .	0.7111	28.89	49.1
" " " + 0.5% Bile . .	0.7242	27.58	46.9
" " " + 1.0% " . .	0.7283	27.17	46.2
" " " + 5.0% " . .	0.7662	23.38	39.7
" " " + 10.0% " . .	0.7854	21.46	36.5
" " " + 20.0% " . .	0.7819	21.81	37.1

**Experiment 52.** Neutral extract of ox pancreas. Fresh ox bile.

In this experiment the proteids of the pancreatic extract were not treated with acid, but sufficient acid was added to the fibrin to saturate it, or half saturate it (as tested by tropæolin oo), prior to addition of the pancreatic extract.

<i>Conditions.</i>	<i>Undigested residue.</i>	<i>Fibrin digested.</i>	<i>Relative proteolytic action.</i>
Neutral Fibrin . . . . .	0.3643 gram	63.57 per cent	100.0
Fibrin saturated with acid (5 c.c. 0.2% HCl),	0.5755	42.45	68.3
“ “ “ + 10% Bile . . .	0.5490	45.10	70.9
“ half “ “ (2.5 c.c. 0.2% HCl),	0.4690	53.10	83.5
“ “ “ “ + 5% Bile . . .	0.4980	50.20	78.9

In only one of these experiments (Experiment 47) do we see any distinct suggestion of aid to pancreatic proteolysis when bile or bile salts are added to a pancreatic extract containing combined acid. Combined acid alone tends to retard proteolysis, and the addition of bile to such mixtures as a rule increases still further the extent of retardation. Our results afford no confirmation whatever of the view that bile greatly aids pancreatic juice in its proteolytic action on acid fibrin. Neither are we inclined to believe “that pancreatic juice, plus bile, plus hydrochloric acid, can accomplish more work in proteolysis than can any other known pancreatic mixture.”<sup>1</sup> If such were the case we fail to see why some evidence of such favorable action should not appear in our results. The inhibitory action of acids alone, and of acids and bile combined, on pancreatic proteolysis is not in our judgment to be looked upon as unfavorable to the normal digestive processes of the small intestine. What right have we to assume that the conditions existent in the normal duodenum are such as to require pancreatic proteolysis to take place in the presence of acid, either free or combined? The combined or free acid which passes from the stomach through the pylorus is without doubt quickly removed by absorption or destroyed by neutralization. The evidence is certainly in favor of the view that the contents of the duodenum are generally alkaline. This question has been admirably discussed in a recent paper by Moore and Rockwood,<sup>2</sup> in which also a large number of experimental data are offered, showing that in many animals at least, under different forms of diet, the contents of the intestine from pylorus to cæcum react alkaline. In some cases, to be sure, the contents closely adjacent to the pylorus were

<sup>1</sup> RACHFORD and SOUTHGATE: *loc. cit.*

<sup>2</sup> MOORE and ROCKWOOD: *Journal of physiology*, 1897, xxi, p. 373.

found to be acid, but when this was the case the acidity was usually limited to a few inches. Hence, we are inclined to believe that pancreatic proteolysis as it occurs in the normal intestine takes place, to a great extent, in the presence of a neutral or alkaline reaction, and that under such conditions the proportion of bile ordinarily present is not inimical to the process.

## THE REINFORCEMENT OF VOLUNTARY MUSCULAR CONTRACTIONS.

By ALLEN CLEGHORN, M.D.

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IN 1890 Bowditch and Warren<sup>1</sup> found that the knee-jerk was accelerated or reinforced when the interval between the various sensory stimuli used in their experiments and the blow on the patellar tendon was less than three-tenths second; when this interval exceeded three-tenths second an inhibitory or negative result was obtained. Their results were so uniform that it seemed desirable to ascertain whether sensory stimuli would exert a similar influence upon voluntary muscular contractions, and to that end the following experiments were undertaken.

The subject of the experiment contracted his muscles rhythmically every three seconds. The signal for contraction was the sound of a metronome beating every half second. The contractions were recorded by means of Mosso's ergograph and were registered on a revolving drum. Each contraction was communicated by the recording lever of the ergograph to a Czermak electric double lever. The reader may be reminded that this most useful piece of mechanism (Fig. 1) was designed to record the limits of oscillation of a moving body without being otherwise affected by the movements of the body. By it an electric current can be either made or broken at the moment when the movement has reached its full extent. It consists of two levers (C) and (I) (Fig. 2), each connected with one pole of a battery (F). Both levers move on the same axis (H). The first lever (C) is U-shaped and freely movable. It embraces the second lever. The latter (I) is stationary, except

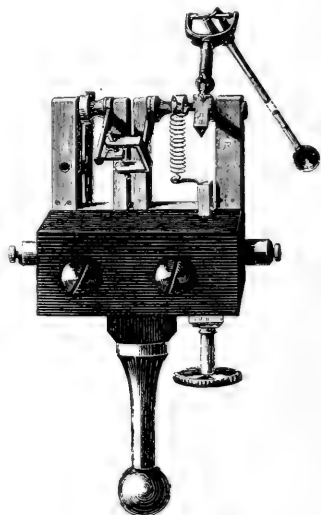


FIGURE 1.

<sup>1</sup> BOWDITCH and WARREN : *Journal of physiology*, 1890, xi, p. 25.



when either elevated or depressed by the action of the first. Each arm of the first lever can be made either a conductor or a nonconductor by means of small screws (B and C), tipped with platinum or ivory respectively. This lever is connected with the ergograph (K)

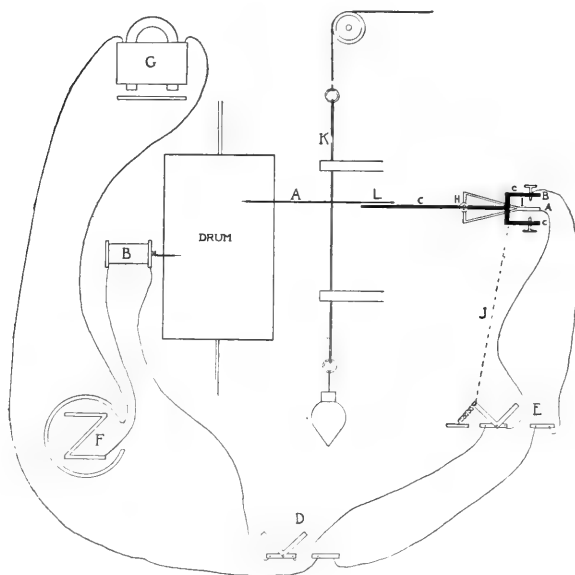


FIGURE 2. Diagram of the double-lever and the recording apparatus.

at L. A movement of the recording arm (A) of the ergograph (K), which is connected with the long or "U" lever (C) at L, brings one of the arms of the "U" into contact with the indifferent, *i. e.*, stationary lever I. Should this be the arm carrying the ivory-pointed screw no connection is made, but if it be the arm with the platinum-pointed screw the current is at once completed. By means of this instrument and an automatic short-

circuiting key (E), which it was subsequently found necessary to introduce, the stimuli were controlled in a definite manner. Thus, when A was depressed or in a position of rest, *i. e.*, the position it occupied when the muscles were completely relaxed, C was raised, through its connection at L, and made to turn on the axis at H, and so to pull on the cord (J) attached to the key E and hence to short-circuit itself automatically. Without this arrangement unavoidable vibrations would have broken the circuit through the sensitive contact of a and b and thus have caused the signal marker (B) and the electro-magnet (G) to act. With this arrangement, however, when A began to ascend, C was lowered and contact made between a and b, while the spring at E pulled open the short-circuiting key, the cord J between C and E being relaxed, and so allowed the current to pass a—b; consequently the ivory-tipped screw c—by elevating C and so drawing b away from a—broke the circuit

as soon as the recording lever (A) of the ergograph (K) began to descend.<sup>1</sup>

The stimuli used in these experiments were light flashed into the eye, a sudden sound, and induction shocks applied to the skin. For the retinal stimulus a flash from a thirty-two candle-power electric lamp was suddenly brought to bear on the eyes, which were in darkness, by means of Czermak's lever, the subject at the beginning of his muscular contraction breaking the current<sup>2</sup> and so by means of the electro-magnet (G) releasing a catch which let fall the shutter of the box containing the electric lamp. The sound stimulus was effected in the same way, except that the breaking of the circuit released a hammer which struck upon a tin disk connected with the ears by a stethoscope. Similarly, in the case of the stimulation of the skin, the breaking of the circuit opened the short-circuiting key in the secondary circuit of a du Bois-Reymond inductorium, hammer in action, and so let a series of induction shocks pass to the subject through common sponge electrodes fastened to the left arm (the right made the contractions). The point at which the reinforcing stimuli were made was indicated on the drum by an electric signal marker. The subject usually contracted against the weight of two kilogrammes. The electric current was under the immediate control of the operator, by means of the short-circuiting key (D), which cut out the action of Czermak's lever and the automatic key (E); consequently no stimulus could take place except at the will of the operator, for the circuit could not be broken by Czermak's lever unless D was open. The operator closed the circuit at irregularly varying intervals so that the subject might not know when the stimulus was to be applied. Fig. 2 illustrates the electrical connections for the three stimuli of sound, light, and the induction shock.

It was found that a sensory stimulus applied just as the muscle was beginning to contract, caused an increase in the height of the contraction (Fig. 3).

At this point in the research Hofbauer<sup>3</sup> published experiments similar to my own. He used sound as the reinforcing stimulus and

<sup>1</sup> For a full account of the double lever see CZERMAK, J. H.: *Der electrische Doppelhebel*, Leipzig, 1871.

<sup>2</sup> The contact screws of Czermak's lever were here reversed, the lower one (c) being the conductor; the previous description applies to the arrangement in the experiments to be described presently.

<sup>3</sup> HOFBAUER: *Archiv f. d. ges. Physiol.*, 1897, lxxviii, p. 546.

obtained practically the same results. In both researches the augmentation was particularly noticeable as fatigue set in and the contractions grew smaller.

The most important feature noticed in my experiments was the fact that the relaxation following a stimulated contraction was de-

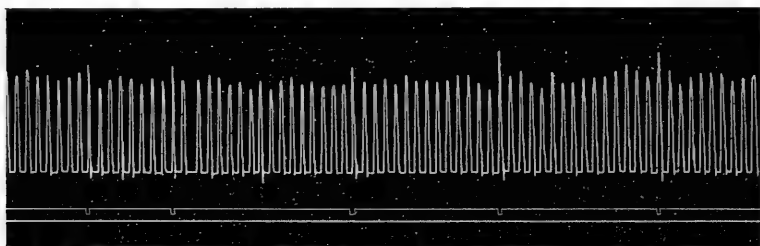


FIGURE 3. One-fourth original size. Reinforcement of voluntary contraction by sensory stimuli. The sensory stimulus was applied at the breaks in the horizontal line beneath the contractions. The load was one kilo.

cidedly quicker and more complete than that following a normal or unstimulated one, even when the stimulated contraction showed no signs of augmentation, and as Hofbauer's paper fully covered the ground of the augmented contractions, attention was now turned to the relaxation phenomena. Czermak's levers were now so arranged that the reinforcing stimulus should be made at the moment the subject began to relax from a contraction.<sup>1</sup> With this arrangement it was found that the duration of relaxation is very considerably shortened when the subject is stimulated at the moment of relaxation, as the accompanying tracings show. So marked was this phenomenon that whereas the average time of a whole unstimulated or normal muscle-curve was about one second, the time of a contraction reinforced at the moment of relaxation averaged only 0.65 second, the difference (0.35 second) being taken from the time of relaxation. The stimulated relaxation is not only quicker than the normal but also more complete; the end of a normal relaxation is slow; usually it exhibits one or two very long curves before reaching the base line, which it approaches in a very gradual manner. Relaxation under the influence of the stimulus, on the contrary, shows nothing of this, but is

<sup>1</sup> The contact screw was changed from c to b, c being now the non-conductor; thus electric contact was preserved during the contraction period but broken as soon as relaxation began. See the description of the apparatus and its connections on page 337.

a sudden sharp drop directly to the base line and sometimes beyond it. Fig. 4 shows this clearly, the broken-line curve being the stimulated one.

In the course of the research it was noticed that the relaxation of some of the subjects was quicker than that of others, while the height of their normal contraction varied also, some persons giving a strong, high contraction while others gave a small, feeble one. Again, some reacted in a more decisive way than others, and in a few instances the subject gradually became accustomed to the stimuli and reacted more slowly than at first. In some cases, finally, it was observed that the next contraction following the reinforced one exhibited the same phenomenon (see Fig. 5), but this occurred in a manner too irregular to enable conclusions to be drawn from it.

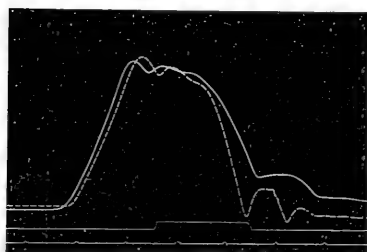


FIGURE 4. One-fifth original size. The curves record voluntary muscular contractions (load, one kilo). In the broken curve, during the period marked by the rise in the line just above the time record, a faradic current was applied to the skin. The breaks in this curve were painted in after the curve was drawn. The lowest tracing gives the time in fifths of seconds.

The table on page 341 shows at a glance the effect of the various stimuli on muscular relaxation.

Each of the three parts of the table is compiled from one tracing, in each case taken from a different individual, and is typical of the results obtained from all the other tracings. The same number of normal and stimulated contractions are given in order to facilitate comparison. In the first section of the table light was the reinforcing stimulus; it was applied at the beginning of muscular *relaxation*. From this portion we can see that in a normal muscular movement the period of muscular relaxation is slightly longer (equal in two cases) than the time of contraction, while in the movements with simultaneous retinal stimulation the reverse is the case, the time of relaxation being considerably shorter than the time of contraction (see Fig. 3). With sound as the stimulus, the same results are noticed, the shortening of the relaxation time being again marked. The same effect is gained by a simple cutaneous sensory stimulus (electric). No attempt was made to find the results of using powerful shocks.

*The duration (in seconds) of voluntary muscular contraction and relaxation (1) without simultaneous stimulation, and (2) with simultaneous visual, auditory, or cutaneous stimuli at the beginning of relaxation.*

LIGHT.				SOUND.				INDUCTION SHOCKS TO THE SKIN.			
No stimulus.		Stimulus at beginning of relaxation.		No stimulus.		Stimulus at beginning of relaxation.		No stimulus.		Stimulus at beginning of relaxation.	
Contraction.	Relaxation.	Contraction.	Relaxation.	Contraction.	Relaxation.	Contraction.	Relaxation.	Contraction.	Relaxation.	Contraction.	Relaxation.
.5	.7	.45	.35	.4	.5	.4	.2	.4	.5	.4	.4
.5	.5	.55	.2	.4	.5	.4	.3	.4	.5	.45	.3
.5	.6	.45	.35	.5	.5	.6	.4	.4	.55	.35	.35
.5	.7	.5	.3	.35	.45	.5	.25	.3	.6	.5	.3
.55	.55	.5	.25	.5	.5	.45	.3	.4	.4	.5	.3
.51	.61	.49	.29	.43	.49	.47	.29	.38	.51	.44	.33
AVERAGES.											

It appears, therefore, that the phase of relaxation after voluntary muscular contraction is shortened by a sensory stimulus applied at the beginning of relaxation.

I pass now to a discussion of the cause of this interesting phenomenon. It is necessary to inquire first whether the shortening of the relaxation phase (or acceleration of relaxation) is due to a reflex contraction of the extensors of the forearm, now relaxing from their contraction as antagonists during the contraction of the flexor muscles. Such a reflex contraction of the extensors would forcibly elongate the relaxing flexors and thus shorten their phase of relaxation.

The solution of this new problem required that the contractions of both extensors and flexors should be registered simultaneously, — a difficult task, as it proved, necessitating an addition to the apparatus already in use. The wrist being fixed as before in Mosso's ergograph, the subject flexed his arm so as to bring the upper arm at right angles to the forearm. Two upright supports firmly screwed to the base board of the ergograph embraced the elbow and upper arm, which were then tightly bandaged in this position, the bandage including the uprights and the upper arm so that no portion of the

limb except the finger in connection with the ergograph could move. The button of a Marey receiving tambour was placed in contact with the integument over the extensors, the tambour itself being supported from below. Connection was now made with a recording tambour, the lever of which carried a Pflüger's marker which wrote vertically on the drum; this did away with the difference that would result were one curve drawn by a lever moving perpendicularly and the other (extensor) curve drawn by a lever which moved in the arc of a circle.

Over twenty different subjects were examined, and rather more than five hundred contractions were taken from each. The tracings obtained by this method all showed the same characteristics and demonstrated the fact that the antagonistic extensors and the flexors relax in the same way. In other words, the stimulus seemed to affect

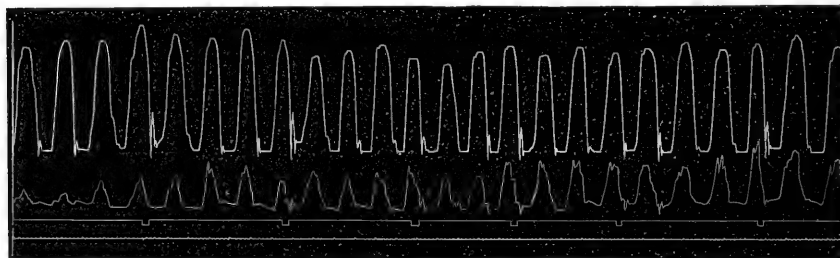


FIGURE 5. One-fourth original size. The uppermost curve was drawn by the flexors of the forearm. The second curve was drawn by the extensors. The breaks in the third curve indicate the stimuli. The lowermost curve is the time in fifths seconds.

the extensors in a similar manner to the flexors. Figure 5 shows the action of both sets of muscles under the influence of the reinforcing stimulus of sound. The tracing is especially valuable as the subject was contracting against the weight of only one kilo, thus minimizing any influence which the weight might have on the relaxation of the muscles. In this tracing the points of the recording levers were accurately placed in the same vertical line. It is seen from these results that the acceleration of the relaxation phase cannot be explained by the reflex contraction of the extensors of the forearm.

A second explanation suggests itself. The relaxation phase in voluntary contraction may perhaps be regarded as the gradual subsidence of the contraction-discharge from the motor neurons. If such a process were inhibited by the stimulation of the retina, the auditory

apparatus, or the skin, the flexor muscles would at once cease to oppose the extending force exerted by the load which they had raised. The passive muscles would then be rapidly extended by the load, and the relaxation curve would fall more steeply, as actually observed. Instances of the reflex inhibition of motor discharges in consequence of sensory excitation are sufficiently numerous. The interesting experiment of Bubnoff and Heidenhain<sup>1</sup> may be cited as an example. These observers found that in some stages of morphine poisoning the subminimal electrical excitation of a cortical motor field produces strong tonic contractions lasting for some time. If a sensory stimulus, such as gently stroking the skin, or blowing on the face, is now applied, the muscles at once relax. The contraction process in the central neurons is in this case cut short by the sensory stimulus. Attractive as this explanation of the acceleration of relaxation witnessed in my experiments may be, there are reasons which seem to preclude its acceptance. An examination of Fig. 5 will show that a muscle weighted with one kilo relaxed as quickly as one weighted with three kilos. Had the contraction process been suddenly inhibited, the extension of the still partially shortened flexors — now no longer in receipt of the motor discharge from the central neurons — would have been more rapid with the greater load. In my experiments, an increase in the load was not followed by an increase in the rapidity of relaxation.

If the quickening of relaxation is not to be explained by the contraction of antagonists or by the passive extension of the muscles in consequence of the reflex inhibition of the contraction process in the phase of sinking energy, — and these explanations cannot be reconciled with my results, — there seems to be no way of avoiding the conclusion that the quickening is due to the augmentation of an active relaxation process. That the phase of sinking energy in the contraction process is as much an active conversion of stored power as is the phase of rising energy has been long considered probable. Indeed, instances are not wanting of a modification of the contraction process by the stimulation of peripheral nerves. Richet<sup>2</sup> and Biedermann<sup>3</sup> have thus relaxed the contracted muscles of a crayfish claw and the

<sup>1</sup> BUBNOFF and HEIDENHAIN: *Archiv f. d. ges. Physiologie*, 1881, xxvi, p. 137.

<sup>2</sup> RICHET: *Cong. périod. internat. d. sc. méd. Compt. rend.*, 1879, Amst.; 1880, vi, p. 554-560.

<sup>3</sup> BIEDERMANN: *Sitzungsber. der königl. Acad. der Wissensch. zu Wien*, 1887, xcv, p. 7.

sartorius muscle of a dog to which veratrine had been given. Kaiser<sup>1</sup> tetanized the nerve of a muscle that had been brought into a state of tonic contraction by exciting its nerves with glycerine, and observed relaxation. Wedensky<sup>2</sup> finds that an ordinary nerve muscle preparation may be made either to relax or to contract according to the strength of the stimulus employed. Many other instances could be cited. Further, we possess ample evidence that cerebral influences may directly cause relaxation in muscular tissue. A familiar example is the action of the inhibitory fibres of the vagus on the heart. Muscular relaxation has even been obtained by direct cortical stimulation. Thus Bubnoff and Heidenhain<sup>3</sup> found that stimulation of the motor fields of contracted muscles, and indeed of other fields as well, would produce relaxation. Sherrington and Hering<sup>4</sup> have also obtained muscular relaxation by stimulating cortical motor areas. It is with these experiments that my own results should probably be placed, for they show that in the human subject sensory stimuli modify reflexly the relaxation from voluntary muscular contraction as well as the contraction itself.

The result of my experiments, then, favors the view that the acceleration of relaxation is due to the augmentation of an "active" relaxation process, rather than to the inhibition of the contraction process, but it would perhaps, in the present state of knowledge, be unsafe to make too positive a statement regarding the nature of the phase of sinking energy in muscular contraction.

In conclusion I desire to express my appreciation of the many valuable suggestions of Professor Bowditch, under whose direction this work was done.

#### SUMMARY.

1. A sensory stimulus applied at the beginning of a voluntary contraction increases the height of the contraction.
2. The relaxation following a contraction with intercalated sensory stimulus is quicker and more complete than when no stimulus is given.
3. This acceleration of relaxation is not due to augmentation of the contraction of the antagonistic muscles, for the relaxation of the ex-

<sup>1</sup> KAISER: *Zeitschrift für Biologie*, 1891, xxviii, p. 423.

<sup>2</sup> WEDENSKY: *Archives de physiologie*, 1891, iii, p. 687.

<sup>3</sup> BUBNOFF and HEIDENHAIN: *loc. cit.*

<sup>4</sup> SHERRINGTON and HERING: *Archiv f. d. ges. Physiol.*, 1897, lxviii, p. 222.



tensors does not visibly differ in rapidity and extent from the relaxation of the flexor muscles.

4. The acceleration of relaxation cannot be ascribed to the sensory stimulus inhibiting the discharge from the motor neurons and thus permitting the rapid passive extension of the muscles by the load of the ergograph, for the acceleration does not increase with the increase of the load.

5. In the present state of knowledge, the acceleration is best explained as an augmentation of an active relaxation process by the sensory stimulus.

## ON CERTAIN CHARACTERISTICS OF THE PRESSURE SENSATIONS OF THE HUMAN SKIN.

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BY pressure sensations are to be understood those sensations which are provoked by non-painful mechanical stimuli applied to the skin. It appears that such stimuli must produce a local deformation of the skin in order to be effective. In Meissner's experiment of immersing the hand in mercury of the temperature of the skin, a considerable amount of pressure is exerted upon the skin of the submerged part, but no sensations are there provoked. In this case there is sufficient pressure to stimulate, but, a large surface being subjected to the same pressure, there is no deformation and consequently no sensation.

Von Frey<sup>1</sup> has shown that mechanical stimuli of threshold strength are felt only at the moment of their application, the continuation and end of the stimulus not being perceived; that is, the sensation vanishes very soon after the application of the weight, and the removal of the weight is not noticed. He has shown, further, that stimuli above the threshold strength may be felt as continuing sensations, although the intensity of the sensation diminishes, the unloading being always more difficult to perceive than the loading. The sensation may outlast the stimulus, probably as a result of the deformation of the skin, which remains for some time. Finally, von Frey has demonstrated that the effectiveness of the stimulus depends upon certain factors in addition to the strength of the stimulus; namely, upon the size and position of the surface stimulated and the rapidity with which the stimulus is applied.

In order to determine more clearly the value of the physiological factors involved, von Frey devised test hairs (*Reizhaare*), which furnish a very circumscribed mechanical stimulus, capable of gradation, and thus measure quite accurately the number, position, and relative

<sup>1</sup> von FREY: *Abhandl. d. math. - physikal. Cl. d. königl. Sächsischen Gesellsch. d. Wissensch.*, 1896, xxiii, p. 175.

sensitiveness of the points designated by Blix<sup>1</sup> as "pressure points." These "test hairs" consist of short pieces of hair, preferably from the human head, glued by one end at right angles to the end of a small stick. For each hair two measurements are made; a micrometer measurement of the diameter of the cross-section, from which the area of the cross-section is calculated; and the weight which the hair can lift when its free end is brought to bear on a scalebeam. The later measurement determines the "power" of the hair. It has been found that approximately the maximal power can be obtained without undue bending of the hair, and further, that the area of the cross-section and the power remain quite constant for long periods of time. The "pressure" of the hair is its power per unit of surface, that is, the quotient of its power divided by the area of its cross-section. Variety in the sectional surface of the different hairs used and variety in the power of different hairs of the same sectional surface can be obtained by taking pieces of hair of different length.

By means of the test hairs it is found that the sensations of pressure are provoked only at certain so-called "pressure points" which are constant in their location but which vary in the number distributed to equal areas of skin. The pressure points are therefore separated by intervals which are not sensitive to this form of stimulus, and these intervals increase in size as the sensitive points are more scattered. The pressure points are fatigued by rapidly repeated stimuli, but soon regain their normal sensitiveness when left to themselves. Upon the haired portion of the skin, estimated to amount to about 95 per cent of the whole, it has been found that the pressure points correspond to the number of the hairs, the point being located over the hair sac not far from the spot where the hair pierces the epidermis; — the hairs as a rule grow obliquely out of the skin. Such pressure points are subject to stimulation by movements of the hairs — which act as levers — as well as by the deformation produced by the direct contact of objects with the surface of the skin.

With such small surfaces as those of the test hairs it has been observed that physiological effectiveness is not proportional to the amount of pressure used. Test hairs of greater surface and power are more effective than those of smaller surface and less power notwithstanding their hydrostatic pressure may be the same. Test hairs, the power of which is proportional not to the surface of the applied end, but to the radius of that surface, are found to be of equal physi-

<sup>1</sup> BLIX: *Zeitschrift für Biologie*, 1884, xx, p. 141.

ological value. From this it is assumed that the organs thus stimulated are not superficial in their location but lie somewhat deeper, the effect of the deformation upon the deeper lying structures being more marked when the surface over which the pressure is applied is increased. With larger surfaces than those of the test hairs the deformation always attains its maximum effect at the deeper levels of the skin, and then the physiological effectiveness of the deformation-producing stimulus becomes proportional to its pressure.

During the summer of 1897 I had the opportunity of co-operating with Professor von Frey in the Physiological Institute of the University of Leipzig in some extension of the investigations along the line on which he has been engaged, and I gratefully acknowledge my indebtedness to him.<sup>1</sup> The first object of the research thus jointly undertaken was to determine whether deformation caused by traction (*Zug*), — which is opposite in direction to that produced by pressure (*Druck*), — excites the same organs that have been shown to be called into action by pressure, or whether the skin contains also organs which react to traction. It is evident that external objects in contact with the skin produce chiefly the deformations of pressure rather than those of traction, but a brief consideration will show that the tissues of the skin may be subject to pressure, or to changes of pressure, by movements of the underlying structures of the motor apparatus, and that the terminal organs of the so-called pressure sense may be thereby excited. The skin presents a very uneven surface, here convex, there concave, as it conforms to the varying contour of the bones and muscles over which it is stretched. A change of position of these structures must necessarily change the natural tissue-pressure in the overlying skin, increasing that of convex areas when their convexity is increased, and diminishing it when their convexity is diminished: increasing that of concave areas when their concavity is decreased and diminishing it when their concavity is increased. Variations in pressure corresponding to those caused by the deformation of local traction as well as that of local pressure from external objects, but due to body movements, may therefore play a rôle in exciting the nerve organs of the skin, and the impulses thus provoked may be assumed to contribute to our knowledge of the position and the condition of a part, a function ascribed by some to “common sensibility.”

<sup>1</sup> The chief results of our observations have been briefly reported by von Frey to the königliche Sächsische Gesellschaft der Wissenschaften in Leipzig (*Berichte*, Aug. 2, 1897).

Our observations were directed to very small and to large surfaces on the left wrist and thumb, and to the effects of momentary and continued stimuli of different degrees of intensity. The results are given first for very small surfaces, then for large surfaces.

**Very Small Surfaces.**—The method of investigation was as follows:—The pressure points on the triangular non-haired area at the distal end of the anterior surface of the left forearm (which we may term for the sake of brevity the left wrist) were very carefully sought out by the aid of suitable test hairs. Each point was then marked with a minute drop of silver nitrate solution. The physiological character of the surface to be stimulated was thus determined so far as the pressure sense is concerned, and the stimulus could be applied as desired directly to the chosen sensitive point or points, or, as the pressure points in this particular locality are widely scattered, to a non-sensitive area. In order to ensure fixation of the surface to be tested and at the same time the comfort of the person upon whom the tests were to be made (the "Reagent," as we shall term him), the left forearm was held in a plaster of Paris form moulded to fit the forearm from the elbow to the tips of the fingers, leaving its anterior surface exposed sufficiently to allow the forearm to be drawn out and inserted at will. The stimuli were applied by means of a double-arm thin wooden lever 20 or 30 centimetres in length, the axis of which was supported by a heavy and practically immovable stand. The arms of the lever being of unequal length, the lever was brought into equilibrium by a rider placed upon the shorter and remote arm. A light straw attached at right angles to the end of the longer arm served to transmit its movements to the surface of the skin to which the stimulus was to be applied. The surface of the free end of the light straw, the area of which was 0.3 mm.<sup>2</sup> to 0.5 mm.<sup>2</sup>, was glued to the skin, various kinds of adhesive substances being used (Le Page's "Liquid Glue," Collodion, etc.). Weighting the longer arm of the lever—that towards the skin—served to produce pressure; weighting the shorter arm served, on account of the adhesion of the straw, to produce traction. Disturbing oscillations of the lever, and the consequent rapidly changing deformations of the skin, when the weights were brought to bear upon one or the other of the arms of the lever, were avoided by suspending the weights by rubber bands tied to the lever arms. Sometimes two equal weights were suspended at the same time from each arm of the lever at equal distances from its axis, the lever being

thus left in equilibrium, and one weight was allowed to produce its effect by quickly raising the other with the hand. During the tests care was taken to avoid all external disturbance, and the Reagent sat with closed eyes in the most comfortable position possible, attentive to the locality of the skin to be stimulated. The word "now" warned him when a stimulus was about to be applied, and the attention was then especially concentrated.

Two protocols of such tests follow, one having been made upon an isolated sensitive pressure point, the other between previously located points upon a surface not sensitive to pressure: —

June 4, 1897. **Reagent C.** End of straw glued upon a sensitive pressure point on the volar side of the left wrist. Surface loaded: 0.3 mm<sup>2</sup>.

Time. h. m.	Actual weight in grams.	Reply of Reagent to Stimulus of	
		Pressure.	Traction.
12 28	10	Pressure (after 20 sec.) weaker; (after 30 sec.) vanished.	
30	4	. . . . .	Pressure, stronger than before; remains.
42	2	. . . . .	Nothing.
....	4	. . . . .	Traction?
54	10	Pressure, continuing, not strong; after 30 sec., vanished.	
....	2	. . . . .	Touch, vanishes very quickly.
57	10	Touch, weak, only momentary.	
....	4	. . . . .	Pressure, rather strong, soon diminishing, after 20 sec. apparently gone, then again perceived, finally definitely vanished.
1 1	4	. . . . .	Touch, very weak; after a few seconds, gone.
....	10	Touch (better sensation of deformation), stronger than before, lasting longer (about 20 sec.).	
....	4	. . . . .	Touch, continues some seconds.
....	10	Traction, probably; after 20 sec., gone.	
....	6	. . . . .	Pressure? Not strong.

June 5, 1897. **Reagent C.** End of straw glued upon a space between three pressure points on the volar side of the left wrist. Surface loaded: 0.3 mm<sup>2</sup>.

Time. h. m.	Actual weight in grams.	Reply of Reagent to a Stimulus of	
		Pressure.	Traction.
11 51	2	Touch, momentary but distinct.	
....	2	. . . . .	Touch, the same strength but lasting longer.
54	10	Pressure, distinct and lasting ; after 20 sec., weaker ; after 35 sec., uncertain ; after 50 sec., nothing.	
....	2	. . . . .	Touch, momentary pressure.
12 00	2	Touch, momentary.	
....	4	. . . . .	Touch, momentary, weaker than before.
....	2	Touch, momentary, somewhat more distinct.	
....	2	. . . . .	Touch, pressure distinct but momentary.
....	10	Touch, distinct and lasting ; after 10 sec., weaker ; after 20 sec., vanished.	
....	....	. . . . .	Touch, very momentary and somewhat weak.
24	2	. . . . .	Touch, momentary; after 30 sec., gone.
....	2	Touch, momentary, but as strong as before.	
....	5	. . . . .	Pressure, continuing, but not long ; after a few seconds, gone.

From the foregoing and other similar tests it was found that the so-called pressure points shown to be sensitive to the deformation caused by pressure are equally sensitive to the deformation caused by traction, and, what is most striking, it was seen that with very small surfaces there is inability to determine the direction of the deformation, that is, to distinguish between pressure and traction, the sensation being that simply of a deformation even with strengths of stimulus which are very marked and the action of which is long continued. Pressure and traction each produced comparatively quick fatigue, the duration of the sensation falling markedly

short of that of the stimulus, and the removal of the stimulus being unperceived.

Attention was next turned to the determination of the effect of fatigue produced by a long continued pressure stimulus upon momentary traction stimuli following immediately after the removal of the fatiguing load. The stimulus used to produce fatigue consisted of a 400 gram weight hung upon the pulley of the axis of the lever. The distance of the weight from the axis was  $\frac{1}{40}$  that between the axis and the straw by which the load was transmitted to the skin. Thus the actual weight upon the tested surface was 10 grams. The sectional area of the end of the straw being 0.5 mm.<sup>2</sup>, the actual pressure was 20 grams to the square millimetre. The momentary stimuli were obtained by means of small double-arm thin wooden levers arranged at right angles to the main lever already described and so placed that they could be made to strike upon it on each side of and at equal distances (*e. g.*, about  $\frac{1}{10}$  the lever length) from its axis. These levers were moved by weights hung upon the pulley of the axis on the side towards the main lever, and the height of their stroke was determined by an adjustable screw in a post placed under the remote arm of each lever to serve as a stop. This arrangement permitted the selection of a stroke that could provoke a weak or a distinct sensation as desired; and, by depressing the remote arm till it touched the stop and then suddenly releasing it, any number of uniform momentary stimuli could be applied.

An isolated, sensitive pressure point was selected and the end of the straw glued upon it. The following protocols set forth the results obtained from two series of tests in which momentary stimuli of pressure and then of traction were applied, the fatigue in both cases being caused by pressure.

The results of such tests as those here given in detail show that with very small surfaces repeated momentary pressure or traction stimuli of a uniform strength just above the threshold value always excite a similar sensation, if the pause between successive stimuli is sufficient to avoid fatigue. The results show also that if a strong pressure-producing stimulus be allowed to act sufficiently long to produce fatigue and then removed and momentary rhythmical stimuli immediately applied, the latter are not at first perceived, but soon begin to be felt, although imperfectly, and with an increasing distinctness inconstant in degree. Several minutes may elapse before the



sensations regain their original uniform strength. It was shown further that the fatigue produced by pressure is as effective in im-

July 3, 1897. **Reagent C.** End of straw glued upon a very sensitive pressure point on the radial side of the left wrist. Surface loaded: 0.5 mm<sup>2</sup>. Fatiguing stimulus 20 grams mm<sup>2</sup>. Momentary or "stroke" stimuli as near threshold strength as possible.

A. — Momentary or stroke stimulus applied as a pressure stimulus.		
Time. h. m. sec.	Stimulus.	Statements of Reagent.
	Stroke stimulus applied several times.	Felt each time, but weak.
11 3 00	Fatiguing stimulus applied.	
30	. . . . .	Weaker.
4 00	. . . . .	Uncertain.
30	. . . . .	Nothing.
6 00	Fatiguing stimulus removed.	Nothing.
	Stroke stimulus applied.	No.
6 15	. . . . .	Uncertain.
30	. . . . .	No.
45	. . . . .	No.
7 00	. . . . .	Yes, very weak.
15	. . . . .	Uncertain.
30	. . . . .	Yes, weak.
45	. . . . .	Uncertain.
8 00	. . . . .	Yes, weak.
15	. . . . .	Yes, weak.
30	. . . . .	No.
45	. . . . .	No.
9 00	. . . . .	Yes, weak.
15	. . . . .	No.
30	. . . . .	Yes.
45	. . . . .	Yes, very weak.
10 00	. . . . .	Yes.
15	. . . . .	Yes.
30	. . . . .	Uncertain.
45	. . . . .	Yes, weak.
11 00	. . . . .	Yes.
15	. . . . .	Yes, it becomes more distinct.
30	. . . . .	Yes.
45	. . . . .	Yes.
12 00	. . . . .	Yes, quite constant and distinct.

July 3, 1897. Reagent C. — Continued.

## B. — Momentary or stroke stimulus applied as a traction stimulus.

Time. h. m. sec.			Stimulus.	Statements of Reagent.
			Stroke stimulus applied several times.	Felt each time, weak but distinct, about as strong as pressure stimulus in A.
11	28	00	Fatiguing stimulus applied.	
	29	30	. . . . .	Not completely vanished.
	30	30	. . . . .	Uncertain, variable.
	31	00	Fatiguing stimulus removed.	Nothing.
			Stroke stimulus applied.	Uncertain.
	15		. . . . .	No.
	30		. . . . .	No.
	45		. . . . .	Uncertain.
32	00		. . . . .	No.
	15		. . . . .	No.
	30		. . . . .	No.
	45		. . . . .	Uncertain.
33	00		. . . . .	Yes, but very weak.
	15		. . . . .	Yes, a little more distinct.
	30		. . . . .	Very weak.
	45		. . . . .	Yes.
34	00		. . . . .	Yes, still weak.
	15		. . . . .	Uncertain.
	30		. . . . .	Yes.
	45		. . . . .	No.
35	00		. . . . .	Yes.
	15		. . . . .	Yes.
	30		. . . . .	Yes.
	45		. . . . .	Uncertain.
36	00		. . . . .	No.
	15		. . . . .	No.
	30		. . . . .	Yes.
	45		. . . . .	Yes.
37	00		. . . . .	Yes.
	15		. . . . .	Yes.
	30		. . . . .	Yes.
	45		. . . . .	Yes.
38	00		. . . . .	Yes.

pairing the sensations provoked by subsequent momentary traction stimuli as those called out by pressure stimuli of the same strength. Therefore the pressure which causes fatigue for pressure also causes an equal fatigue for traction.

**Large Surfaces.**—It has been shown thus far that with very small surfaces points most sensitive to pressure are also most sensitive to traction; that their sensitiveness to each kind of stimulus is approximately the same; that fatigue produced by pressure is fatigue for subsequent traction stimuli as well as for pressure stimuli; and that even with strong and continued stimuli producing deformation in one or the other direction there is inability to determine the direction of the stimulus, that is, to distinguish between pressure and traction. Tests were now made upon larger surfaces. A cork disc with a sectional area of 50 mm.<sup>2</sup> was slipped on to the free end of the straw used in the foregoing tests and glued upon the surface to be tested. Observations were first made to determine the effect of momentary stimuli applied to the skin upon the most convex part of the ball of the left thumb. Such stimuli were obtained by the "stroke levers" arranged as in the foregoing tests and could be changed at will from pressure to traction. These observations were followed by others with continued stimuli of different strengths.

Following is a protocol: —

July 7, 1897. **Reagent F.** End of cork glued on the convex surface of the ball of the left thumb. Surface loaded: 50 mm<sup>2</sup>. Very strong **momentary** stimuli by "stroke levers."

Time. h. m.	Kind of Stimulus.	Statements of Reagent.	Time.	Kind of Stimulus.	Statements of Reagents.
11 25	Pressure.	Yes.	Intervals 5 to 10 sec.	Pressure.	Yes.
Intervals 5 to 10 sec.	Traction.	Yes, the same.		Traction.	Strong.
	Traction.	Yes.		Pressure.	Strong.
	Pressure.	Yes.		Traction.	Strong.
	Pressure.	Weak.		Pressure.	Perhaps weaker.
	Traction.	Strong.		Traction.	Strong.
Pressure and traction not distinguished.					

**Continued stimuli.** Instead of "stroke levers," weights were hung on main lever four-tenths of its length distant from its axis. Duration of stimulus from 5 to 10 seconds, with intervals of several seconds.

With 100 grams.			
With 100 grams acting as pressure or traction according to the pleasure of the observer and without previous information to the person upon whom the observations were made, 10 such stimuli were each correctly judged as to the direction of the stimulus.			
With 20 grams.			
Kind of Stimulus.	Statements of Reagent.	Kind of Stimulus.	Statements of Reagent.
Pressure.	Uncertain.	Traction.	Traction.
Traction.	Perhaps traction.	Pressure.	Traction, uncertain.
Traction.	Perhaps traction.	Pressure.	Also traction.
Pressure.	Pressure.	Traction.	Also traction.
Pressure.	Uncertain.	. . . .	. . . . .

Repeated tests of the above character made on both of us upon the wrist as well as upon the thumb and with stimuli of different strengths, showed that with large surfaces momentary stimuli of pressure and traction even of marked strength, provoking distinct sensations, could not be distinguished as to their direction, a deformation undetermined in character being in each case alone perceived. When stimuli were continued instead of momentary it was found that with smaller weights, that is, with a diminution in the strength of the stimuli, the ability to judge of the character of the deformation was also impaired.

In order to obtain equality in the rapidity of application of the continued stimuli and thus eliminate the influence of that factor, which had previously been shown to be an important one in the effectiveness of pressure stimuli, the following arrangement of the apparatus already described was made. The weights hanging on rubber bands attached to each side of the axis of the main lever were supported by the two levers previously used as "stroke levers" but now placed under the main lever. The ends of the arms that supported the weights rested upon a shelf attached to a horizontal clock-work kymograph drum. Rotation of the drum carried the ends of the levers down and

allowed the weights which they supported to act upon the main lever. By placing a sufficiently heavy rider upon the remote arm of either lever that lever could be held in place so that only one weight would be brought to act upon the main lever when the drum was set in motion. The excursion of the drum was limited to about one-fourth revolution. The stimulus was removed by turning the drum back by the hand to its former position, the shelf lifting the end of the lever and thereby the weight. A counterpoise clamped on the opposite side of the drum served to offset the effect of the weights upon the shelf when the drum was at rest and a stimulus was not being applied. In the above manner a continued pressure or traction stimulus of any desired strength could always be applied with the same rapidity.

A protocol of a test made under such conditions is presented below. The tests showed clearly that a certain degree of strength is essential to a correct judgment of the kind of deformation produced by continued stimuli upon large surfaces. By gradually increasing the strength of stimulus, the size of the surface stimulated and the rapidity of application of the stimulus remaining constant, it was found that the ability to distinguish the direction of the deformation appeared with a certain increase of weight, not suddenly but gradually, some still undetermined difference in the character of the stimulus being first noticed. With stimuli of sufficient strength a correct judgment as to their direction could always be formed:—

July 16, 1897. **Reagent F.** End of cork glued upon the convex surface of the ball of the left thumb. Surface loaded: 10 mm<sup>2</sup>. Continued stimuli 30 grams, two-tenths lever length from axis of main lever, corresponding to an actual load of 6 grams. Duration of stimulus, 10 seconds.

Time. h. m.	Kind of Stimulus.	Statements of Reagent.
10 37	Pressure.	Distinct excitation, continuing; not determinable whether pressure or traction, perhaps traction.
41	Traction.	The same, but weaker, lasting a very short time.
45	Traction.	More distinct, does not last very long; believe it is pressure. More distinct than second stimulus, about as long as first.
50	Pressure.	Distinct, lasting; different from the preceding excitation, perhaps traction.
55	Pressure.	Distinct and lasting; cannot say what it is.
11 00	Traction.	Weaker, very weak, almost momentary.

30 grams, pressure side 2 spaces from axis; actual load 6 grams.

30 grams, traction side  $2\frac{1}{2}$  spaces from axis; actual load 7.5 grams.

Time. h. m.	Kind of Stimulus.	Statements of Reagent.
11 15	Traction.	Quite distinct, not very strong, impossible to distinguish.
18	Pressure.	Distinct, lasting; not determinable whether pressure or traction, perhaps traction.
22	Pressure.	Distinct, lasting, gradually vanishing; perhaps pressure, quite uncertain.
27	Traction.	Distinct, lasting; not determinable.
31	Traction.	Yes, distinct and lasting; perhaps pressure.
34	Pressure.	Exactly the same as to intensity and character.
<p>All stimuli notably of equal strength.</p> <p>(It is to be noted that the removal of the stimulus was not perceived, and that the sensation outlasted the stimulus.)</p>		

#### CONCLUSIONS.

Collectively the tests show that the recognition of the direction of a deformation, that is, the ability to distinguish between pressure and traction, depends upon the size of the surface stimulated, the duration of the stimulus, and the strength of the stimulus. The perception of a deformation is therefore a simpler psychological process than the recognition of its character. The impulses provoked by variations in pressure appear to contain in themselves no determination of the direction of the exciting deformation. That determination is gained by a combination of impulses of some intensity, of more than momentary duration, and arising from a not too limited area of the skin.

Our demonstration that the points most sensitive to pressure are equally sensitive to traction; that the impulses produced by stimuli in either direction provoke simply sensations of deformation without indications as to its direction; that a stimulus which causes fatigue for pressure stimuli causes also fatigue for traction stimuli; and that the strength of the stimulus, the rapidity of its application, and the size and the location of the surface to which it is applied, influence equally the effectiveness of traction and of pressure stimuli — makes it probable that the organs in the skin which are stimulated by pressure are also stimulated by traction.

# THE MOVEMENTS OF THE STOMACH STUDIED BY MEANS OF THE RÖNTGEN RAYS.<sup>1</sup>

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	Page
I. Introductory literature . . . . .	360
II. The method . . . . .	362
III. The anatomy of the stomach and its relations to the shadow . . . . .	364
IV. The normal movements of the stomach . . . . .	365
1. Movements of the pyloric part . . . . .	367
2. Movements of the pyloric sphincter . . . . .	368
3. Activity of the cardiac portion . . . . .	370
V. The movements of the stomach in vomiting . . . . .	373
VI. The effect of the movements of the stomach on the food . . . . .	375
VII. Salivary digestion in the stomach . . . . .	379
VIII. The inhibition of stomach movements during emotion . . . . .	380

SINCE the stomach gives no obvious external sign of its workings, investigators of gastric movements have hitherto been obliged to confine their studies to pathological subjects or to animals subjected to serious operative interference. Observations made under these necessarily abnormal conditions have yielded a literature<sup>2</sup> which is full of conflicting statements and uncertain results. The only sure conclusion to be drawn from this material is that when the stomach receives food, obscure peristaltic contractions are set going, which in some way churn the food to a liquid chyme and force it into the intestines. How imperfectly this describes the real workings of the stomach will appear from the following account of the actions of the organ studied by a new method. The mixing of a small quantity of subnitrate of bismuth with the food allows not only the contractions of the gastric wall, but also the movements of the gastric contents

<sup>1</sup> The first account of this work was given at the meeting of the American Physiological Society, in May, 1897 (see *Science*, June 11, 1897); and the later results were presented at the meeting of the Society in December, 1897. A summary of the results was published in the *Proceedings of the American Physiological Society*, this *Journal*, 1898, i, p. xiii. A report of the research was also made to the *Boston Society of Medical Sciences*, February 15, 1898.

<sup>2</sup> POENSGEN (*Die motorische Verrichtungen des menschlichen Magens und ihre Störungen*, Strassburg, 1882,) gives a comprehensive review of the literature to that date.

to be seen with the Röntgen rays in the uninjured animal during normal digestion. An unsuspected nicety of mechanical action and a surprising sensitiveness to nervous conditions have thereby been disclosed.

## I. INTRODUCTORY LITERATURE.

The early writings on the subject of gastric movements are characterized by general inferences from physical laws and from the anatomical structure of the stomach. According to Galen,<sup>1</sup> the stomach had four functions: to draw the food from the mouth (*facultas attractrix*), to retain the food (*facultas retentrix*) during the process of chemical digestion (*facultas alteratrix*), and, finally, to pass the changed material onward (*facultas expultrix*). In later writings the *facultas attractrix* failed to appear as one of the functions of the stomach. Fallopius,<sup>2</sup> in the sixteenth century, changed the notion of the *facultas retentrix* by suggesting that the pylorus alone performed this office, and that the muscles of the gastric wall could help only by remaining quiet. Thus the *facultas alteratrix* and the *facultas expultrix* are left as true gastric functions. It is with the latter activity and its effects that this paper is concerned.

The ideas of the early writers concerning the pylorus and cardia are of interest. The cardia, they were agreed, is closed during normal digestion in order to keep the food from re-entering the œsophagus. The pylorus they looked upon as the ruler of the actions of the stomach. Such names as pylorus (keeper of the gate), janitor justus, and rector, which the first investigators gave to the sphincter, indicate their theories of its functions. The passage of chyme into the duodenum, the keeping of undigested food in the stomach, the act of vomiting, were all dependent, they believed, on the "will" of the pylorus.<sup>3</sup>

No substantial advance was made beyond these hypotheses until the beginning of the eighteenth century, when Wepfer and Schwartz applied the experimental method to the study of the gastric movements and laid the foundation of a more accurate knowledge. Wepfer<sup>4</sup> vivisected wolves, dogs, and cats, and observed constrictions following stimulation of the stomach. He remarked a general con-

<sup>1</sup> GALEN: Opera omnia. Leipzig, 1822, iii, pp. 275, 281.

<sup>2</sup> FALLOPIUS: Opera omnia; observationes anatomicæ. Frankfort, 1600, p. 412.

<sup>3</sup> VAN HELMONT: Opera omnia. Frankfort, 1707, p. 215.

<sup>4</sup> WEPFER: Historia cicutæ aquaticæ. Basel, 1679, p. 152 *et seq.*



traction of the pyloric part in vomiting (pp. 152, 168, and 250), and noted peristaltic and antiperistaltic movements passing over the organ. About the middle of the stomach he frequently saw a deep constriction. The investigations of Schwartz<sup>1</sup> are more valuable in that his search was for the normal action of the muscular coats. The movements, as he observed them, were generally only slight. They began either at the pylorus and passed to the left, half-way to the cardia, or started at the fundus and went to the pylorus. The contractions and relaxations, following one another, formed larger or smaller depressions and elevations, *i. e.*, more or less definite waves.

Near the middle of the last century, Haller,<sup>2</sup> after confirming the results obtained by Schwartz and Wepfer, summarized his knowledge of the motor functions of the stomach as follows: In general, contraction alternates with relaxation, so that the stomach is, now here, now there, made narrower by longitudinal or transverse depressions; then in these same places relaxation and bulging occur (pp. 260–262, and p. 276). So long as both apertures are closed the food is driven hither and thither by the shifting movements. It first takes a definite direction when the cardia or the pylorus opens. If the cardia opens, there is an antiperistalsis followed by regurgitation and vomiting (p. 281). If, on the contrary, the pylorus relaxes, a contraction, starting at the œsophagus, pushes the contents of the stomach into the duodenum. The pylorus allows the passage of fluids, but if it be stimulated by over distention or by hard pieces of food, it closes tightly (p. 277).

Such was the knowledge of gastric movements in Haller's time. A comparison of his descriptions with those in any standard work on physiology published ten or fifteen years ago will show that, despite very many researches, little advance had been made. Examinations of animals and men with gastric fistulas, studies of the stomach through the atrophied abdominal wall, and vivisection, have yielded numerous results, but these have not been harmonious, and have led to much controversy. Prominent in this mass of material as a valuable contribution are Beaumont's careful observations through the gastric fistula of Alexis St. Martin. Beaumont's work has recently been confirmed by Hofmeister and Schütz, who, with Rossbach, Hirsch, Openchowski, and others, have presented during the last twelve years

<sup>1</sup> B. SCHWARTZ in Haller's *Dissertationes anatomicæ*. Göttingen, 1746, i, pp. 337–338.

<sup>2</sup> HALLER: *Elementa physiologiæ*. Berne, 1764, vi, p. 260 *et seq.*

much new and interesting information. Since, however, it will conduce to clearness to set forth the results of these investigations in connection with my own work, their consideration will be deferred until later.

It will then appear that these later investigations, like the earlier researches, disagree as to the details of the stomach movements. Such differences in results are the proper outcome of the abnormal conditions under which the studies have been conducted. Obviously, in order to see the natural movements of the stomach, the organ should be observed in its natural state, and not after it has been disturbed by removal from the abdomen or by the adhesions and losses of substance incident to gastric fistulas.

As a means of watching the gastric motor activities under normal circumstances, Dr. H. P. Bowditch, in the autumn of 1896, suggested the use of the Röntgen rays. The present paper is the result of the work thus far completed. The kind assistance and stimulating counsel of Dr. Bowditch throughout the investigation are gratefully acknowledged.

## II. THE METHOD.

The method consists in mixing subnitrate of bismuth—a harmless, non-irritating powder—with the food, and observing the movements of the swallowed mass by means of the Röntgen rays. As is now generally known, the picture thrown on the fluorescent screen by the Röntgen rays is one of shadows of varying intensity; the denser the substance, the darker the shadow. There is nothing in the structure of the stomach to cause it to cast a different shade from that of its neighboring organs. But the dense bismuth powder, uniformly mixed with the food that fills the stomach, throws the dark shadow of the stomach contents on the screen, and the changes in the shape of the outlines indicate the intrinsic movements of the organ.

The animal used throughout the research was the cat. The meal given before making an observation consisted of from fifteen to eighteen grams of dry bread, softened to a mushy mass by milk, hot water, or thin gravy, and mixed with from one to five grams of subnitrate of bismuth, according to the purpose in hand. One or two grams of the bismuth compound produce a dim shadow of the stomach within which may be clearly seen the darker forms of food containing a larger amount of the substance; three grams are enough for ordinary observations; four or five grams are needed to

see the passage of food from the pylorus. The cat was usually kept from eating for at least twelve hours before an observation, in order that the stomach might be wholly free from contents transparent to the X-rays.

The construction of the holder on which the cat was tied is shown in the diagram (Fig. 1). It consisted of a framework supporting a sheet of black cotton cloth. The frame was made of two side pieces each 80 cm. long and 2.5 cm. square, connected at either end by blocks 2.5 cm. thick, 12.5 cm. wide, and 16 cm. long. The black cloth, which sagged for the comfort of the cat, was held by strips of wood nailed to the inner face of the frame. Through the side pieces were holes 0.6 cm. in diameter, and 5 cm. apart. Each of the leather nooses securing the legs went down through one of these holes, and up through another, in which it was made fast by forcing a pointed peg into the hole with it. The cat's head was held by two pegs, one on either side of the neck, joined above by a leather thong. One of the pegs was movable and could be put in any of the three holes, 3, 4.5, or 6 centimetres from the other peg, according to the thickness of the cat's neck.



FIGURE 1.

For seeing the regular movements of the stomach the cat was tied back downward, with the fore paws in nooses at either side, and with the hind legs stretched out and fastened to the holder at the cat's right. For watching the passage of food from the pylorus, the hind legs were both fastened to the left side of the frame, so that the cat lay on her left flank. Most of the female cats would lie on the holder by the hour without making any attempt to break away or manifesting any signs of discomfort. In marked contrast was the behavior of the males. Almost without exception they seemed worried when fastened down. The interesting effects of these different ways of reacting to novel surroundings will be described later.

The cat-holder was supported at either end. Below it at a distance of 19 cm. was placed the tube generating the Röntgen rays. This tube had a self-regulating device for maintaining a uniform vacuum, —very useful in that it allowed long observations with rays of uniform intensity. A Töpler-Holtz machine, run by a small motor, produced the electrical discharge through the tube. This apparatus

was placed behind the holder. The light from the tube and the machine was shut off from the observer by drapings of black cloth, so that in the dark room where the work was carried on it was possible to use an open fluorescent screen with sides only two centimetres high. This plan was found especially valuable in that it permitted tracing the outlines of the stomach on tissue paper laid over the fluorescent surface.

### III. THE ANATOMY OF THE STOMACH AND ITS RELATIONS TO THE SHADOW.

It must be constantly borne in mind that the shadows described in this research are cast by the gastric contents, — not by the stomach itself. Therefore the movements of the organ are not seen directly, but are indicated by their effect on the contained food. Variations in the length and breadth of the stomach can be inferred from changes in the outline of the shadow, but variations in the front-to-back diameter of the organ must be judged from changes in the intensity of the shadow.

The form of the active stomach soon after food has been taken is shown in outline in Figure 2. Since the several parts of the stomach are to be mentioned frequently, it will be well to recall them here in their relations to the outline. The larger, cardiac part of the organ lies to the left of a line through *w x*. Into it the œsophagus opens

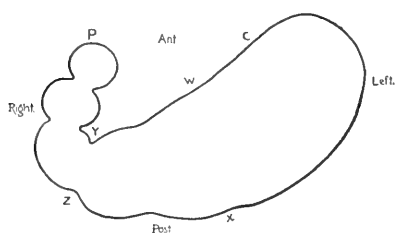


FIGURE 2.

through the cardiac sphincter, or cardia, at *c*. The pyloric part, which includes all of the stomach situated at the right of a line *w x*, is closed by the pylorus at *p*. This part has two divisions; the antrum at the right of the line *y z*, and the preantral part of the pyloric portion, or middle region of

the stomach, between the lines *w x* and *y z*. The lesser curvature corresponds approximately to the anterior border of the shadow *c w p*; the greater curvature to the more extensive sweep, *c p*, along the posterior border.

The wall of the cat's stomach consists of three coats, but as this paper deals only with the functions of the muscular coat, that alone will be described. The gastric muscular fibres are disposed in three sets: an outer longitudinal layer, a middle circular layer, and a set

of inner oblique fibres. The longitudinal fibres continue those of the œsophagus, and, radiating over the cardiac end, become more marked along the curvatures than on the front and back surfaces. Over the antrum they lie in a thick, uniform layer. The circular fibres form a complete investment, and are arranged in rings at right angles to the curved axis of the stomach. Towards the pyloric end they become denser and stronger, and at the pylorus form a thick bundle, the pyloric sphincter. Separating the antrum from the rest of the stomach, at *y z*, is a special thickening of the circular fibres, called by the early writers<sup>1</sup> the "transverse band," and described by Hofmeister and Schütz<sup>2</sup> as the "sphincter antri pylorici." The oblique fibres start from the left of the cardiac orifice, and pass as two strong bands along the anterior part of the dorsal and ventral surfaces, giving off fine fasciculi to the circular musculature; towards the antrum they gradually disappear.

The musculature of the stomach consists of smooth muscle fibres, the chief physiological characteristics of which are slowness of contraction, rhythmic alternation of contraction and relaxation, and a very great tonicity, or power of prolonged contraction. The action of these muscles in the process of gastric digestion is now to be considered.

#### IV. THE NORMAL MOVEMENTS OF THE STOMACH.

Since the time of Haller the chief contributors to the knowledge of the mechanics of the stomach have been Beaumont, Hofmeister and Schütz, and Rossbach.

Beaumont's famous investigations on Alexis St. Martin are recorded in almost all general works on physiology. Through a gastric fistula he introduced a thermometer-tube and observed how it was affected by the motions of the stomach. His conclusions are as follows: "The circular or transverse muscles contract progressively from left to right. When the impulse arrives at the *transverse band*, this is excited to a more forcible contraction, and, closing upon the alimentary matter and fluids contained in the pyloric end, prevents their regurgitation. The muscles of the pyloric end, now contracting upon the contents detained there, separate and expel some portion of the chyme. . . . After the contractile impulse is carried to the pyloric

<sup>1</sup> BEAUMONT: Physiology of digestion. Burlington, 1847, p. 104.

<sup>2</sup> HOFMEISTER and SCHÜTZ: Archiv für exper. Pathol. und Pharmakol., 1886, xx, p. 7.

extremity, the circular band and all the transverse muscles become relaxed, and a contraction commences in a reversed direction, from right to left, and carries the contents again to the splenic extremity to undergo similar revolutions." <sup>1</sup>

In close accord with Beaumont's description of the activities of the human stomach are the records of the investigations on the stomach of dogs by Hofmeister and Schütz.<sup>2</sup> They removed the stomach from the body and placed it in a moist chamber, kept at body-heat and covered with glass. Under such conditions the organ remained active for from sixty to ninety minutes. A typical movement is described by these observers as composed of two phases. In the first phase a constriction of the circular fibres, deeper on the greater curvature, starts a few centimetres from the cardia and passes towards the pylorus. As the constriction proceeds it increases in strength until a maximum is reached about two centimetres in front of the antrum. This annular contraction, called by Hofmeister and Schütz the "preantral constriction," closes the first phase. Immediately thereafter the strong sphincter antri pylorici, or transverse band, contracts. Now, while the preantral constriction is relaxing, the sphincter antri pylorici tightens still more, and the antrum is shut off from the rest of the stomach. As soon as this has occurred a general contraction of the muscles of the antrum follows. Relaxation begins at the sphincter antri pylorici and progresses slowly toward the pylorus; it is sometimes accompanied by an antiperistaltic movement.

Although Rossbach<sup>3</sup> also used dogs, his results vary considerably from those of Hofmeister and Schütz. This discrepancy is possibly accounted for by a difference in method, for Rossbach left the stomach in the body. The dogs were treated with morphia and curare, and the abdomen was then widely opened, so that the movements could be clearly seen. When the stomach was full Rossbach saw deep constrictions begin near the middle and pass in waves to the pylorus. At first these movements were weak: later, however, they became more vigorous. The fundus remained in tonic contraction about its contents and took no part in the peristalsis.

Before attempting to explain the difference in the records of these observers I shall give an account of what may be seen in a cat by use of bismuth subnitrate and the Röntgen rays.

<sup>1</sup> BEAUMONT: *loc. cit.*, p. 106.

<sup>2</sup> HOFMEISTER and SCHÜTZ: *loc. cit.*, p. 1.

<sup>3</sup> ROSSBACH: *Deutsches Archiv für klinische Medicin*, 1890, xlv, p. 296.

**I. Movements of the Pyloric Part.** — Within five minutes after a cat has finished a meal of bread, there is visible near the duodenal end of the antrum a slight annular contraction which moves peristaltically to the pylorus: this is followed by several waves recurring at regular intervals. Two or three minutes after the first movement is seen, very slight constrictions appear near the middle of the stomach, and, pressing deeper into the greater curvature, course slowly towards the pyloric end. As new regions enter into constriction, the fibres just previously contracted become relaxed, so that there is a true moving wave, with a trough between two crests. When a wave swings round the bend in the pyloric part the indentation made by it deepens; and as digestion goes on the antrum elongates and the constrictions running over it grow stronger, but, until the stomach is nearly empty, they do not entirely divide the cavity. After the antrum has lengthened, a wave takes about thirty-six seconds to move from the middle of the stomach to the pylorus. At all periods of digestion the waves recur at intervals of almost exactly ten seconds. So regular is this rhythm that many times I have been able to determine within two or three seconds when a minute had elapsed simply by counting six similar phases of the undulations as they passed a given point. It results from this rhythm that when one wave is just beginning, several others are already running in order before it. Between the rings of constriction the stomach is bulged out, as shown in the various outlines in Figures 3, 4, and 5. The number of waves during a single period of digestion is larger than might possibly at first be supposed. In a cat that finished eating fifteen grams of bread at 10.52 A.M., the waves were running regularly at 11.00 o'clock. The stomach was not free from food until 6.12 P.M. During that time the cat was fastened to the holder at intervals of half an hour and the waves were always observed, following one another in slow and monotonous succession. At the rate of three hundred and sixty an hour, approximately two thousand six hundred waves passed over the antrum during that single digestive period.

From the above review, it will be manifest that my observations of the movements of the pyloric part agree closely with those of Rossbach, but differ considerably from the harmonious results of the work of Beaumont, and Hofmeister and Schütz. Beaumont's methods, however, may be justly criticised on the ground that the thermometer-tube which he held in the stomach was wholly unlike food and very liable to bring about unwonted contractions in so sensitive an organ

as the stomach. Further, the movements observed by Hofmeister and Schütz, as Ewald has pointed out,<sup>1</sup> may easily have resulted from the abnormal stimulus due to lack of blood — a potent cause of peristalsis. And it will be shown later that the accounts given by these investigators describe very well the actions of the stomach when stimulated by an unusual irritant. In this connection it may be added that since the publication of the preliminary notice of my work,<sup>2</sup> Roux and Bathazard,<sup>3</sup> using the Röntgen rays, have published the results of observations on the stomachs of the dog and man, similar to those thus far described in this paper.

The fact that my observations and those of Roux and Bathazard were conducted under normal conditions, and that the conditions of Rossbach's experiments were more nearly normal than those of the other observers mentioned, warrants the conclusion that the pyloric part has a more important function than that of merely expelling the contents of the stomach into the intestines. After summarizing the description given by Hofmeister and Schütz, Ewald, for *a priori* reasons, declares: "I cannot accept this view. The plain fact that the pyloric portion secretes a strongly digesting fluid containing pepsin and hydrochloric acid, proves it to be an important part for the peptonizing function of the stomach."<sup>4</sup> The account of the remarkable manner in which the pyloric portion performs this function must be deferred until the movements of other parts of the stomach have been considered.

2. **Movements of the Pyloric Sphincter.** — Rossbach<sup>5</sup> mars his otherwise careful work by declaring that the pylorus is tightly closed during the whole digestive period of from four to eight hours; and that then the sphincter relaxes and the peristaltic waves empty the stomach. That this is not the normal action of the sphincter has been shown by several observers. Hirsch<sup>6</sup> watched dogs with duodenal fistulas and saw food come from the stomach at intervals of one-fourth of a minute to several minutes. Roux and Bathazard<sup>7</sup>

<sup>1</sup> EWALD: Lectures on digestion. London, 1891, p. 66.

<sup>2</sup> CANNON: Science, June 11, 1897, p. 902.

<sup>3</sup> ROUX et BATHAZARD: Comptes rendus de la soc. de biologie, 1897, 10 S, iv, pp. 704, 785, and Archives de physiologie, 1898, 5 S, x, p. 85.

<sup>4</sup> EWALD: *loc. cit.*, p. 67.

<sup>5</sup> ROSSBACH: Deutsches Archiv für klinische Medicin, 1890, xlv, p. 317.

<sup>6</sup> HIRSCH: Centralblatt für klin. Medicin, 1892, xiii, p. 994.

<sup>7</sup> ROUX et BATHAZARD: Comptes rendus de la soc. de biologie, 1897, 10 S, iv, p. 705.



maintain that in dogs food enters the duodenum at the completion of each wave of constriction. Observations on the cat, however, do not support their view, but agree rather with the statement of Hirsch.

In cats fed with bread mixed with subnitrate of bismuth, ten or fifteen minutes elapse after the first constriction in the antrum before any food can be seen in the duodenum. When food does appear it is spurted through the pylorus and shoots along the intestine for two or three centimetres. Not every constriction-wave forces food from the antrum. On one occasion, about an hour after the movements began, three consecutive waves were seen, each of which squirted food into the duodenum. The pylorus remained closed against the next eight waves, opened for the ninth, but closed once more against the tenth and eleventh. For each of the four succeeding waves the sphincter relaxed, but blocked the food brought by three constrictions that followed; and in this irregular way the food continued passing from the stomach. Near the end of gastric digestion, when the constrictions are very deep, it may be that the pylorus opens for every wave.

When a hard bit of food reaches the pylorus, the sphincter closes tightly and remains closed longer than when the food is soft. This action of the sphincter was shown by giving with the regular food of the cat a dry, hard pellet of equal parts of starch paste and bismuth subnitrate, about the size of a pea. The food itself contained merely enough bismuth to throw a dim shadow, near the centre of which the pellet could be clearly seen as a dark object. The continual passing of the contraction-waves finally brought the little ball to the pylorus. When it arrived there, five grams of bismuth subnitrate were introduced into the stomach through a tube in the œsophagus. This was done in order that the food passing into the intestines after the ball came to the pylorus, might be distinguished from that which had gone on before. By kneading the stomach the bismuth was distributed, as shown by the uniformly black shadow. The pellet could still be seen near the end of the antrum when the constrictions passed over it. Now, although the waves continued to run regularly, the very black food did not gather in the intestines in sufficient amount to be recognized until forty-two minutes after it had been introduced. And when, finally, the food did show itself in the intestines, its shadow contrasted strongly with that of the food which had already passed on. The slowness of the expulsion is not to be regarded as wholly due to the

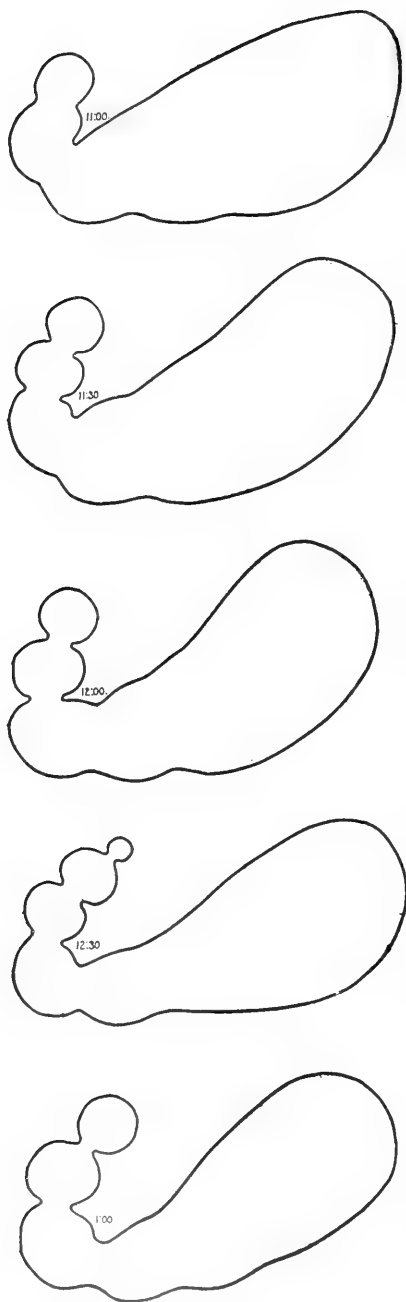


FIGURE 3.

hard mass. No doubt the kneading of the stomach mixed the contents of different parts of the organ and brought to the pylorus food not yet sufficiently digested to be passed by that selective sphincter. But this does not explain the whole delay. Food similar to that given here except that it contained no hard particles has usually been seen as small masses in the intestines within fifteen minutes after being swallowed. A part of the delay was evidently, therefore, caused by the hard pellet. Further evidence on this point was secured when, on one occasion, the sphincter was seen to open only seven times in twenty minutes following the arrival of a hard particle of food at the pylorus. The conclusion may therefore be drawn that hard morsels keep the pylorus closed and hinder the passage of the food into the duodenum.

### 3. Activity of the Cardiac Portion.

—The part played by the fundus apparently has not hitherto been properly appreciated. It has been regarded as the place for peptic digestion or as a passive reservoir for food; but it is in fact a most interestingly active reservoir.

The action of the cardiac portion will be best understood by comparing the appearances the stomach presents at various stages in a digestive period. In order to show these stages I carefully made a set of three tracings of the out-

lines of the stomach as soon as possible after a cat had finished eating, and another set of three every half hour thereafter, until the contents had disappeared (Figs. 3, 4, and 5). These tracings were made by placing white tissue paper over the fluorescent screen, and drawing with a thick lead pencil, easily seen, as much of the boundary of the stomach as I could at the end of each expiration. Between the times for making the drawings the cat was allowed to rest quietly on a mat, but care was taken to lay her in the same position on the holder for every drawing. The drawings of each set were afterwards fastened over one another, so that the lines coincided as closely as possible. Another piece of tissue paper was then put over these, and all four sheets were laid on an illuminated pane of glass. It was thus easy to get a composite tracing, which, considering the movement imparted to the stomach by respiration, and the dimness of the shadows in the later stages of digestion, probably represents more exactly than any single drawing the outline of the stomach for each successive period.

A comparison of these drawings shows that as digestion proceeds the antrum appears gradually to elongate and acquire a greater capacity, and that the constrictions make deeper indentations in it. But when the fundus has lost most of its contents, the longitudinal and circular fibres of the antrum contract to make it again shorter and smaller. Its change of form, however, compared with the rest of the stomach, is slight.

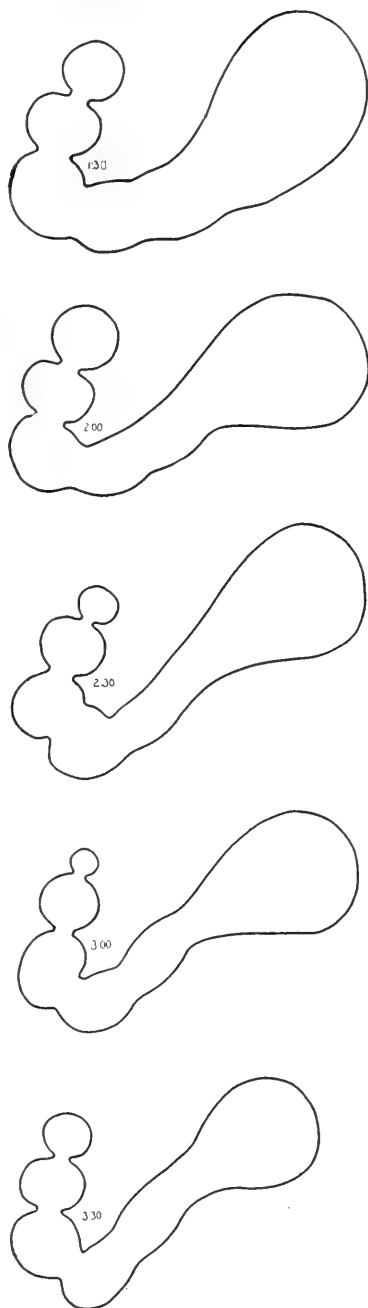


FIGURE 4.

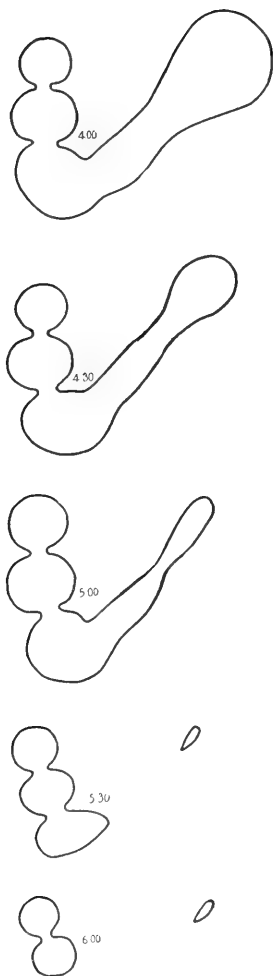


FIGURE 5. — Figures 3, 4, and 5 present outlines of the shadow of the contents of the stomach cast on a fluorescent screen by the Röntgen rays. The drawings were made by tracing the outline of the shadow on tissue paper laid upon the fluorescent surface, and are about one-half the actual size. They show the change in the appearance of the stomach at intervals of half an hour, from the time of eating until the stomach is nearly empty.

The first region to decrease markedly in size is the preantral part of the pyloric portion. The peristaltic undulations, caused by the circular fibres, start at the beginning of this portion, and gradually, by their rhythmic recurrence, press some of the contents into the antrum. As the process continues, the smooth muscle fibres with their remarkable tonicity contract closely about the food that remains, so that the middle region comes to have the shape of a tube (Fig. 4 — 1.30 P. M. to 2.30 P. M.), with the rounded fundus at one end and the active antrum at the other. Along the tube very shallow constrictions may be seen following one another to the pylorus.

At this juncture the longitudinal fibres which cover the fundus like radiating fingers, and the circular and oblique fibres reaching in all directions about this spherical region, begin to contract. Thus the contents of the fundus are squeezed into the tubular portion. This process, accompanied by a slight shortening of the tube, goes on until the shadow cast by the fundus is almost wholly obliterated (Fig. 5 — 5.30 P. M.).

The waves of constriction moving along the tubular portion press the food onward as fast as they receive it from the contracting fundus; and when the fundus is at last emptied they sweep the contents of the tube into the antrum (Fig. 5 — 5.00 P. M. to 6.00 P. M.). Here the operation is continued by the deeper constrictions till finally (in this instance, at 6.12 P. M.) with the exception of a slight trace of food in the fundus, nothing is to be seen in the stomach at all.

The food in the fundus may possibly be

slightly affected by the to-and-fro movements of the diaphragm in respiration. With normal breathing the upper border of the cardiac portion swings through about one centimetre; with dyspnœa, or deep breathing, through one and a half or two centimetres. Since the lower border does not move so much, the contents are gently pressed, and then released from pressure, at each respiration. The pyloric portion is moved very little by the diaphragm, the oscillation being less than a half centimetre.

Moritz<sup>1</sup> has pointed out the value of an organ like the stomach for holding the bulk of the food and serving it out a little at a time so that the intestines may not become congested during their digestive and absorptive processes. All of the advantages supposed to be thus secured to the intestines may be claimed also for the stomach itself. For the preceding description indicates, and experiments to be described later prove, that the stomach is composed of two physiologically distinct portions: the busy antrum, over which during digestion constriction-waves are running in continuous rhythm; and the cardiac part, which is an active reservoir, pressing out its contents a little at a time as the antral mechanism is ready to receive them.<sup>2</sup>

#### V. THE MOVEMENTS OF THE STOMACH IN VOMITING.

The appearance of the stomach during vomiting has been studied particularly by Openchowski.<sup>3</sup> He says that when an emetic is given there follows a quivering of the stomach wall, which, beginning near the pylorus, shows itself later in the antral and middle regions of the stomach. The quivering afterwards passes into a contraction, most strongly marked in the antral part, since the peristaltic waves running down to the antrum from above are continually growing deeper. At the same time the fundus expands spherically. The increased contraction in the pyloric part drives the contents towards the more dilated portion, and thence they are forced into the œsophagus by abdominal pressure.

The same phenomena occur when a cat is given apomorphine hypo-

<sup>1</sup> MORITZ: *Münchener med. Wochenschrift*, 1895, xlii, p. 1146.

<sup>2</sup> By study of the pressure at various parts of the stomach in man, Moritz (*Zeitschr. f. Biologie*, 1895, xxxii, p. 359), and von Pfungen (*Cbl. f. Physiol.*, 1887, i, p. 220), have inferred that the fundus must be quiet and that the motor functions are performed by the pyloric part. Leven has also expressed the same conclusion: *Traité des maladies de l'estomac*, Paris, 1879, p. 16.

<sup>3</sup> OPENCHOWSKI: *Archiv für Physiologie*, 1889, p. 552.

dermatically. First the upper circular muscles relax and become so flaccid that the slightest movement of the abdomen changes the form of the fundus. Then there are apparently irregular twitchings of the fundus wall. Soon a deep constriction starts about three centimetres below the cardia, and, growing in strength, moves toward the pylorus. When it reaches the transverse band the constriction tightens and holds fast, while a wave of contraction sweeps over the antrum. Another similar constriction follows. In the interval the transverse band relaxes slightly, but tightens again when the second wave reaches it. Perhaps a dozen such waves pass; then a firm contraction at the beginning of the antrum completely divides the gastric cavity into two parts. This same division of the stomach into two parts at the transverse band is to be seen when mustard is given. Now, although the waves are still running over the antrum, the whole preantral part of the stomach is fully relaxed. A flattening of the diaphragm, and a quick jerk of the abdominal muscles, accompanied by the opening of the cardia, now force the contents of the fundus into the œsophagus. As the spasmodic contractions of the abdominal muscles are repeated, the gastric wall again tightens around the contained food. Antiperistalsis I have seen only once; then, while the cat was retching, a constriction started at the pylorus and ran back, over the antrum, completely obliterating the antral cavity.

It will be recalled that the principal difference between the movements of the stomach and their effects as described by Beaumont, and Hofmeister and Schütz on the one hand, and Rossbach, Roux and Bathazard, and myself on the other hand, is that the former observed constrictions completely dividing the stomach at the transverse band, and the antrum then squeezing its contents into the intestines; whereas the latter have seen the constrictions moving forward as narrowing rings, but not separating the gastric cavity into two parts.

With the exception of peristalsis in the antrum, the gastric movements at the beginning of emesis are almost exactly the same as those Beaumont, and Hofmeister and Schütz, declare to be the normal contractions of the stomach. Their observations were made, however, when the organ was subjected to unnatural stimulation. In the excised stomach, observed by Hofmeister and Schütz, not only were all nervous connections severed, but likewise all flow of blood to the organ was entirely stopped, and the cutting off of the blood supply is regarded as one of the most powerful predisposing causes of peristal-

tic action.<sup>1</sup> The thermometer-tube used by Beaumont was an irritant to the stomach, as he himself admits. "If the bulb of the thermometer," he writes,<sup>2</sup> "be suffered to be drawn down to the pyloric extremity, and retained there for a short time, or if the experiments be repeated too frequently, it causes severe distress, and a sensation like cramp, or spasm, which ceases on withdrawing the tube, but leaves a sense of soreness and tenderness at the pit of the stomach." Moritz also noticed that a rubber sound introduced into the human stomach proved to be a source of irritation.<sup>3</sup> It seems reasonable to suppose, therefore, that these observers did not see the normal movements, but the actions resulting from abnormal irritation.

## VI. THE EFFECT OF THE MOVEMENTS OF THE STOMACH ON THE FOOD.

In my first observations on the active stomach a bulging of the stomach-wall was to be seen in front of the passing waves. But as food did not immediately appear in the intestine, and as, after the pylorus relaxed, the gastric contents did not diminish rapidly enough to allow the supposition that all of the food squeezed forward by the waves was immediately forced through the pylorus, it was assumed that a part, at least, of the food under pressure was forced back towards the cardia through the constriction-ring. This inference was stated in the preliminary notice of my work.<sup>4</sup> Roux and Bathazard also observed the passage of the undulations over the pyloric part, but state merely that the function of the constrictions is the propulsion of food into the intestine, without mentioning what must be regarded as a very important function, namely, the mixing effect of the waves.

Most writers have agreed that the result of the active and passive movements of the stomach is to force the contents hither and thither, thus mixing them and the gastric juice together. Two observers, Beaumont and Brinton, have attempted to explain the manner of the mixing. Beaumont, after noting how the thermometer-tube, used by him to indicate the gastric motions, was affected, describes the circulation of the food as follows: "The bolus as it enters the cardia turns

<sup>1</sup> MALL: A study of the intestinal contraction. Johns Hopkins hospital reports, i, p. 70.

<sup>2</sup> BEAUMONT: *loc. cit.*, p. 105.

<sup>3</sup> MORITZ: Zeitschr. f. Biologie, 1895, xxxii, p. 369.

<sup>4</sup> CANNON: Science, June 11, 1897, p. 902.

to the left; passes the aperture; descends into the splenic extremity; and follows the great curvature towards the pyloric end. It then returns, in the course of the small curvature, makes its appearance again at the aperture, in its descent into the great curvature, to perform similar revolutions." <sup>1</sup> Brinton <sup>2</sup> bases his theory of the circulation of the food on an analogy between the movement of a constriction over the stomach, and the passage of a septum with a central perforation along the interior of a cylinder full of liquid. The result in both cases, he declares, must be a peripheral current of advance, and a central current of return. Thus in the stomach there would be peripheral currents from the cardia along the walls of the stomach to the pylorus, where they would unite and run as an axial current back to the cardia.

Certain *a priori* objections may be urged against each of these conclusions. In the first place, Beaumont's observations were made on a subject having a gastric fistula, and the adhesions between the stomach and the abdominal wall would prevent the fundus from acting quite normally in relation to its contents. Beaumont's conclusions, furthermore, are based on the movements of a thermometer-tube introduced through the fistula, and on the recognition of particles of food which he had seen before as they passed the fistulous opening: the first method, as has been shown, made the conditions in the stomach more abnormal than they were previously; the second gave uncertain knowledge of the course of the food when out of the observer's sight. Brinton's hypothesis states the probable movements of fluid contents acted on by a passing constriction. But it may be objected that the conditions assumed by him do not exist in all parts of the stomach. For, not only is there no peristalsis visible in the fundus, but with the usual food the fundus contents are not liquid. Moreover, the constrictions at the beginning of the pyloric portion are very slight, and move slowly. The food in front of them is, accordingly, not under much greater pressure than the food behind them. The axial current which might result, therefore, could not be strong enough to go far into the cardiac portion.

It is easily possible to test experimentally the validity of these two theories by watching the action of pieces of food which throw a black shadow in a dimly-outlined stomach. For this purpose little paste pellets of bismuth subnitrate, with starch enough to keep the

<sup>1</sup> BEAUMONT: *loc. cit.*, p. 101.

<sup>2</sup> BRINTON: The diseases of the stomach. Philadelphia, 1865, p. 24.



form, were given with the customary meal. These pellets, it was found, did not break up in the stomach during the gastric digestion of soft bread. Several times I have been fortunate in getting two of the little balls in the axis of the stomach and about a centimetre apart. As the constriction-wave approached them, both moved forward, but not so rapidly as the wave. Now when the constriction overtook the first ball, the ball moved backward through the constricted ring, in the direction of least resistance. The wave then overtook the second ball, and it also passed backward to join its fellow. At the approach of the next wave they were both pushed forward once more, only to be again forced backward, one at a time, through the narrow orifice. As the waves recurred in their persistent rhythm, the balls were seen to be making progress — an oscillating progress — towards the pylorus; for they went forward each time a little farther than they retreated. This to-and-fro movement of the pellets was much more marked in the antrum, where the waves were deep, than in the middle region. On different occasions from nine to twelve minutes have elapsed while the balls were passing from where the waves first affected them to the pylorus; which means that on the way they were moved back and forth by more than a half hundred constrictions.

If the pylorus does not relax, it is evident that a wave approaching it pushes the food into a blind elastic pouch, the only exit from which is through the advancing constricted ring. The constrictions are deeper near the end of the antrum, and the rings are small; consequently the food is squirted back through them with considerable violence. As has been noted, the pylorus opens less frequently for a while after a solid piece of food comes to it. In such a case the slow driving waves squeeze the hard morsel and the soft food about it up to the sphincter, only to have the whole mass shoot back, sometimes half way along the antrum. Over and over again the process is repeated till the sphincter at last opens and allows the more fluid parts to pass. Hofmeister and Schütz, and Moritz have disclaimed any selective action of the pylorus, and declare that solids are driven from the pylorus to the fundus by antiperistalsis. The action of the pylorus which I have seen, however, is more like that described by the earlier investigators; for during digestion there was no antiperistalsis, and the sphincter, separating the fluids from the solids, caused the solids to remain and undergo a tireless rubbing. Frequently when several of these balls have been given at the same time, they

have all been seen in the antrum after the stomach was otherwise empty. Here they remain to be softened in time by the juices or to be forced through the pylorus later, for solids do pass into the intestine. Thus when the teeth neglect their work the stomach attempts to perform their function; the relative inefficiency of the gastric method of grinding and its interference with the normal gastric activities point an obvious hygienic moral.

During the process of digestion the food in the cardiac portion gives no sign of currents. Balls which lie in the fundus immediately after the food is ingested, keep their relative positions until the cardiac portion begins to contract, and then move very slowly towards the antrum. Moreover, the food in the fundus of a cat has the same mushy appearance when examined after gastric peristalsis had been active for an hour and a half that it had when ingested. The contents of the antrum, on the other hand, look quite different and have the consistency of thick soup. The inactivity of the food in the fundus can also be proved by feeding first five grams of bread and bismuth, then five grams without bismuth, and finally five grams again with bismuth in it. The stomach contents are thus arranged in two dark layers along the curvatures, with a light layer between. Tracings made on tissue paper show that ten minutes after peristalsis commenced, the stratification had entirely disappeared in the pyloric part, but that an hour and twenty minutes thereafter the layers were still clearly visible in the cardiac region.

The value of the circulation of the food, as described by Beaumont and Brinton, lay in the supposition that the contents of the stomach were thus brought near to the secreting gastric wall, and that the gastric juice could thus more readily exert its action. Although my observations do not support their theories of mixing currents running throughout the stomach, they still show that the pyloric portion is an admirable device for bringing all of the food under the influence of the glandular secretions of that region. For, when a constriction occurs, the secreting surface enclosed by the ring is brought close around the food lying within the ring in the axis of the stomach. As this constriction passes on, fresh areas of glandular tissue are continuously pressed in around the narrow orifice. And also, as the constriction passes on, a thin stream of gastric contents is continuously forced back through the orifice and thus past the mouths of the glands. The result of this ingenious mechanism is that every part of the secreting surface of the pyloric portion is brought near to every bit

of food, before the latter leaves the stomach, a half hundred times or more, as evidenced by the moving ball.

#### VII. SALIVARY DIGESTION IN THE STOMACH.

The absence of movement in the fundus would seem to give the food during its stay there little opportunity to become mixed with the gastric juices and thus to undergo peptic digestion. The truth of this supposition can easily be proved experimentally by feeding a slightly alkaline meal and later testing the chemical reaction of the contents of various parts of the stomach. A cat which had been without food for fifteen hours was given eighteen grams of mushy bread made slightly alkaline with sodium carbonate. One hour and a half after the cat had finished eating, she was killed and the stomach laid bare by opening the abdomen. A very small hole was then made through the wall in the fundus region, and another similar hole was made into the antrum. By means of a glass pipette food was extracted first from the periphery of the fundus; this food was slightly acid. The cleaned pipette was then introduced two and a half centimetres into the fundus contents and the food thus extracted gave the original alkaline reaction. Specimens of the liquid contents of the antral and middle regions, taken from various depths, were all strongly acid. A dog killed an hour and three-quarters after eating showed similar differences between the reactions of the food in the fundus and the food in the pyloric portion. So, as a matter of fact, the food does not become acid at a uniform rate in all parts of the stomach, as would be the case if Beaumont's and Brinton's theories of mixing currents were true. Moreover, if the facts accorded with their notions, the saliva, which ceases to act in the presence of more than 0.003 per cent free hydrochloric acid,<sup>1</sup> and is destroyed when the percentage of acid proteids is large, would manifestly have its service as a ferment limited to the relatively short time during which the stomach contents, in the process of thorough mixing, were reaching that degree of acidity. There is, however, no movement of food in the fundus, and the alkaline food received from the œsophagus remains alkaline in this region for a considerable period. The nutriment, therefore, if well chewed and thus mixed with saliva, can undergo salivary digestion in the fundus for a considerable period without interference by the acid gastric juice.

<sup>1</sup> CHITTENDEN and SMITH: *Chemical news*, London, 1886, liii, p. 173.

From all these observations the conclusion must be that the fundus acts as a reservoir for the food, in which the digestion of sugars and starches may take place; and that the pyloric portion with its simple but marvellous peristaltic mechanism, by a single process, triturates the food, brings it near to the active glands, stirs it thoroughly with their secretions, and expels the products into the intestines.

#### VIII. THE INHIBITION OF STOMACH MOVEMENTS DURING EMOTION.

Early in the research a marked unlikeness was noticed in the action of the stomachs of male and female cats. The peristalsis seen with only a few exceptions in female cats failed to appear in most of the males, although both had received exactly the same treatment. Along with this difference was a very striking difference in behavior when bound to the holder; the females would lie quiet, mewling occasionally, but purring as soon as they were gently stroked. The males, on the contrary, would fly into a violent rage, struggle to be loose from their fastenings, bite at everything near their heads, cry loudly, and resist all attempts to quiet them. On account of this difference only female cats were used for some time; and the significance at first attributed to the action of the males, was almost forgotten when the following incident recalled it, and suggested that the excitement caused the suspension of the stomach movements. On October 23, 1897, a male cat was fed at 12.00, but was not placed on the holder till ninety minutes later. The waves were passing at the rate of six a minute. The cat fell into a rage and the waves suddenly stopped.

A few days later an observation on a female with kittens explained the absence of gastric movements in the males. While the peristaltic undulations were coursing regularly over the cat's stomach, she suddenly changed from her peaceful sleepiness, began to breathe quickly and struggled to get loose. As soon as the change took place, the movements in the stomach entirely disappeared; the pyloric portion relaxed and presented a smooth rounded outline. I continued observing, and stroked the cat reassuringly. In a moment she became quiet and began to purr. As soon as this happened the movements commenced again in the stomach; first a few constrictions were visible near the end of the antrum, then a few near the sharp bend in the lesser curvature, and finally the waves were running normally from their habitual starting place. By holding the cat's mouth closed between the thumb and last three fingers and covering her nostrils with

the index finger, she could be kept from breathing. At the first sign of discomfort the fingers were removed. This experiment was repeated a great many times on different cats, and invariably the evidence of distress was accompanied by a total suspension of the motor activities of the stomach and a relaxation of the antral fibres.

No amount of kneading or compression of the abdomen with the fingers, short of making the cat angry, would cause the waves to stop; so that the cat's movements, in themselves, were not the source of the inhibition. And since expressions of strong feeling on the part of the animal always accompanied cessation of the constriction-waves, the inhibition was probably due to nervous influence. It has long been common knowledge that violent emotions interfere with the digestive process, but that the gastric motor activities should manifest such extreme sensitiveness to nervous conditions is surprising.

#### SUMMARY.

1. By mixing a harmless powder, subnitrate of bismuth, with the food, the movements of the stomach can be seen by means of the Röntgen rays.

2. The stomach consists of two physiologically distinct parts: the pyloric part and the fundus; over the pyloric part, while food is present, constriction-waves are seen continually coursing towards the pylorus; the fundus is an active reservoir for the food, and squeezes out its contents gradually into the pyloric part.

3. The stomach is emptied by the formation, between the fundus and the antrum, of a tube along which constrictions pass. The contents of the fundus are pressed into the tube and the tube and antrum slowly cleared of food by the waves of constriction.

4. The food in the pyloric portion is first pushed forward by the running wave, and then by pressure of the stomach wall is returned through the ring of constriction; thus the food is thoroughly mixed with gastric juice, and is forced by an oscillating progress to the pylorus.

5. The food in the fundus is not moved by peristalsis and consequently it is not mixed with the gastric juice; salivary digestion can therefore be carried on in this region for a considerable period without being stopped by the acid gastric juice.

6. The pylorus does not open at the approach of every wave, but only at irregular intervals. The arrival of a hard morsel causes the

sphincter to open less frequently than normally, thus materially interfering with the passage of the already liquefied food.

7. Solid food remains in the antrum to be rubbed by the constrictions until triturated, or to be softened by the gastric juice, or later it may be forced into the intestine in the solid state.

8. The constriction-waves have, therefore, three functions: the mixing, trituration, and expulsion of the food.

9. At the beginning of vomiting the gastric cavity is separated into two parts by a constriction at the entrance to the antrum; the cardiac portion is relaxed and the spasmodic contractions of the abdominal muscles force the food through the opened cardia into the œsophagus.

10. The stomach movements are inhibited whenever the cat shows signs of anxiety, rage, or distress.

# CONTRIBUTIONS TO THE PHYSIOLOGY OF THE CARDIAC NERVES IN THE GUINEA-PIG.

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WHILE the guinea-pig has been used largely in experimental pathology, it seems to have been used little as yet in physiological research; in the literature to which I have had access I have noticed but two references to the physiology of the heart or of the cardiac nerves of this animal. In papers published by Legros and Onimus<sup>1</sup> and by MacWilliam<sup>2</sup> references are made to the effect upon the heart of stimulating the vagus nerve in the guinea-pig; it is evident, however, that their experiments upon this animal were very limited in number. The fact that the guinea-pig is used so extensively in experimental pathology may give some interest to the experiments described in this paper.

## I. METHOD OF EXPERIMENTING.

The guinea-pigs used in these experiments were of various sizes; the average weight, however, was 584 grams. Animals of both sexes and of various ages were used.

*Anæsthetics.* The animals were thoroughly anæsthetized in all cases. The anæsthetics used in the various experiments were ether, given by inhalation; chloral hydrate, opium, and morphine, these three given subcutaneously, either singly or in various combinations. When chloral was used alone, 0.5 gram was given a short time before beginning the operation. Smaller doses were given as required during the experiment. Of opium and chloral I have used 1 cubic centimetre of the deodorized tincture of opium combined with 0.25 gram of chloral; of morphine and chloral I have used 0.005 gram of morphine combined with 0.25 gram of chloral. It is usually advisable to give a little ether by inhalation while the dissection is performed.

<sup>1</sup> LEGROS et ONIMUS: *Journal de l'anatomie et de la physiologie*, 1872, pp. 575 and 578.

<sup>2</sup> MACWILLIAM: *Journal of physiology*, 1888, ix, p. 387.

*Recording of blood-pressure.* The blood-pressure was measured by means of a cannula inserted into the proximal stump of one of the carotid arteries. As the arteries of the guinea-pig are very small and the tissues very delicate, the insertion of a cannula into one of the carotids frequently offers difficulties. The following method was found to be of great assistance in performing this operation. After the artery had been dissected from the sheath it was clamped and divided and a fine silk thread was tied to the proximal stump just above the point at which the opening in the artery for the cannula was to be made. The artery was then lifted from the wound and slightly stretched, and the thread attached to the artery was held in the operator's teeth, — a procedure which left one hand free for the use of the forceps and the other to hold the cannula. Moreover, taking the thread in the teeth seems to inhibit the ordinary reflexes of the operator's body and thus he is better enabled to work with a steady hand. The blood-pressure was recorded by a mercurial manometer on the smoked paper of a drum-kymograph, the fineness of the tracing usually obtained being too great for the use of ink to be satisfactory. For excitation of the nerves the faradic current of an ordinary du Bois-Reymond induction coil was used; the current for the primary coil was obtained from an electric-light dynamo, and was maintained at a little less than one ampère, by throwing in the necessary amount of resistance. The interruption of the primary current was by Neef's electro-magnetic hammer. Small shielded electrodes of platinum were used.

*Normal blood-pressure.* The average mean blood-pressure (corrected for the weight of the solution of sodium carbonate) as determined upon guinea-pigs anaesthetized by one or more of the above-mentioned drugs, was 75 millimetres of mercury; the highest blood-pressure observed was 111 mm., the lowest, 56 mm. The average rate of the heart-beat was 200 per minute, the fastest and slowest rates observed being 288 and 132 respectively. The average number of respirations per minute of animals quiet in their cage (the temperature of the room being 20° C.) was 72 per minute.

## II. THE VAGUS NERVE.

The vagi were investigated after exposure in the neck, where they occupy about the same anatomical position in the guinea-pig as in other mammals.



*Section of the vagi.* Section of both vagi in the guinea-pig seems to have little effect upon either the rate of the heart or the blood-pressure. In a few cases slight acceleration was noticed for a short time after the section; but this usually gave place, within ten or twenty seconds, to the normal rate. Much more frequently section was followed by a slight slowing of the rate of the heart during the first ten seconds, this probably being caused by mechanical stimulation of the inhibitory fibres. In no case was a rise of blood-pressure observed to follow section of both vagi. Thus the inhibitory nerves of the heart of the guinea-pig do not seem to be in constant activity.

*Stimulation of the vagus; effect of the season upon the result.* Meyer has shown that the effect upon the heart of stimulation of the vagus nerve is much more marked in the cold-blooded animals than in the warm-blooded ones, and other investigators have confirmed Meyer's conclusions.<sup>1</sup> Since vagus inhibition is less effective in the case of the more frequently beating hearts of warm-blooded animals, the question naturally suggests itself whether in such hearts as that of the guinea-pig, of which the average number of pulsations per minute is 200, the inhibitory power of the vagi is sufficient to bring the heart to a standstill.

My work on the guinea-pig was begun in October, 1896. It was suspended during November and December and resumed in January, 1897. It was continued through January, February, March, and April. For convenience the time may be divided into two periods, an early period, from October until the end of January, and a later period, including the latter part of February, March, and April. During each of these two periods the results of vagus stimulation differed quite markedly from the results obtained during the other period, the curve of inhibition being characteristic of the period in which it was taken. During the first period, *i. e.*, from October to January, cardiac inhibition from excitation of the vagi was much less marked than during the second period from February to April. In no case during the first period was I able to bring the heart to a standstill, no matter how strong a current was applied to the vagus, and I came to the conclusion that in at least one of the warm-blooded animals with a very frequently beating heart, *i. e.*, the guinea-pig, the inhibitory power of the vagus was not sufficient to bring the heart to a standstill. During the first weeks in February, however, I was surprised to find that in one guinea-pig the heart was stopped by a faradic current

<sup>1</sup> See HOUGH: *Journal of physiology*, 1895, xviii, p. 161.

of only moderate strength. For a short time after this I noticed that in some guinea-pigs the heart was stopped by a moderately strong current (the secondary coil being at about 10 cm.), whereas in others the strongest current used (the secondary coil being at 0 cm.) was unable to stop it. During the latter part of February and throughout March and April the heart was invariably brought to a standstill by faradic currents of the strength commonly used in physiological work, *i. e.*, just perceptible when applied to the tongue. In some cases very weak currents, such as are obtained when the secondary coil stands at 24, 28, and 30 centimetres from the primary, sufficed to cause stoppage.

The curves of inhibition obtained during the first period differed in several respects from those obtained during the second period. In the former (see Fig. 1) the slowing of the heart and the fall of blood-

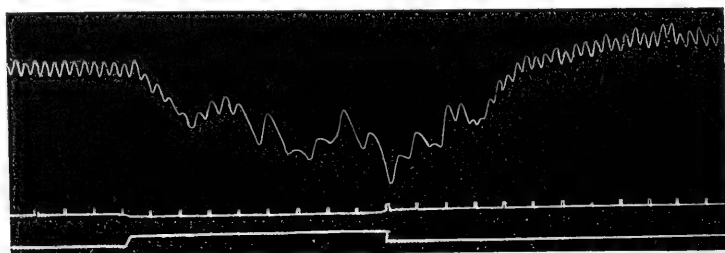


FIGURE 1. Inhibition from stimulation of the left vagus, with the secondary coil at 5 cm. Time in seconds. The line of zero pressure is not shown. The writing-points of the manometer and of the stimulating key were in the same vertical line.

pressure were gradual; the fall of pressure was not very great in proportion to the normal blood-pressure, which as a rule was considerably higher during the first than during the second period. The return of the blood-pressure and the heart-rate to the normal, after the cessation of stimulation, was gradual and fairly regular, and inhibition was followed by comparatively little irregularity of heart-rate or of blood-pressure. On the other hand, during the second period the stopping of the heart and the fall of blood-pressure were sudden, the descent in the curve being sometimes almost vertical (see Fig. 2). The blood-pressure was very irregular after the cessation of stimulation, sometimes failing to return to the ordinary height, while more frequently it rose far above it and then gradually returned to it or fell below it.

At about the season when it was first noticed that excitation of the vagus was bringing the heart to a standstill, it was also noticed that the guinea-pigs under experiment did not live as long as they had formerly done. Later it was noticed that they died very easily, and without any apparent cause; no sooner were the necessary dissections made, and the artery connected with the manometer, than the animal died. Although the anæsthetics were used with great care, and various ones were tried, there was no improvement. At the season in question the dissection was done more carefully and more rapidly than during October, and everything pertaining to the operation was more favorable than during the earlier months; still the animals died before any results of value could be obtained. In many cases the tissues of the neck showed an unduly venous condition of the blood before the cannula was inserted into the artery; respiration ceased; the heart

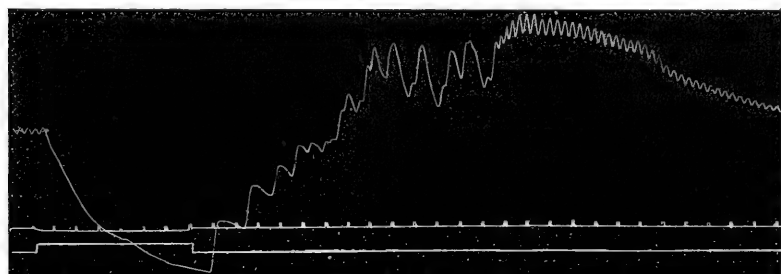


FIGURE 2. Four-fifths the original size. Inhibition from stimulation of the left vagus with the secondary coil at 16 cm. Time in seconds. The line of zero pressure is not shown. The writing-points of the manometer and of the stimulating key were in the same vertical line.

became feeble, and the animal died. The heart usually continued to beat for a short time after respiration had ceased; artificial respiration, however, did not prolong the life of the animal. In April it became practically impossible to get any results whatever from the experiments. In order to be certain that the trouble was not wholly with the special lot of animals on hand, or with the hygienic conditions to which they were exposed, a new lot of animals was procured, but the results were not improved except in a few cases. The only possible explanation of these results seems to be that the lack of vitality was due either to some unknown cause associated with the season of the year, or to the diminution of fresh air and light during the winter months, or to both causes combined. Nearly every one has

experienced in his own person a certain diminution of vitality during the spring months. It is a well-known fact that persons suffering from chronic diseases are much more liable to die during March and April than during other months of the year; and eminent surgeons state that major operations are more liable to be followed by death during those months than at other seasons. There would seem, however, to be some slight evidence in favor of the view that diminution of fresh air and light during the winter months is partially responsible for this lack of vitality in the guinea-pigs, from the fact that, of the last lot of the guinea-pigs used, a few, which had been placed in the sunlight in a specially well ventilated room for two weeks, showed greater vitality than those of the same lot kept elsewhere, although much less vitality than those subjected to experiment from October to January. Hygienic influences alone, however, do not suffice to explain the differences of vitality observed.

It seems clear from what has been said that, in the guinea-pig at least, the efficiency of the stimulation of the inhibitory nerves of the heart is inversely proportional to the degree of vitality possessed by the animal. In a measure this agrees with the opinion expressed by Hough,<sup>1</sup> who states that in his experiments upon cats, when the heart from any cause has been weakened and the pulse has become feeble, stimulation of the vagi has been much more effective than when the heart was vigorous; the slowing of the heart and the fall of blood-pressure being much more pronounced. I did not find, however, that in the guinea-pig when the heart had been weakened from profound anæsthesia, from shock, or from loss of blood, stimulation of the vagi was more efficient, for in no case during the earlier period, from October to January, no matter what was the condition of the animal, did stimulation of the vagi stop the heart. Hough states that in the cat the heart can rarely be brought to a complete standstill, not oftener, I think, than once in twenty cases. I do not know at what season of the year his experiments were made, or in what conditions the animals upon which he operated were kept, but in two of three experiments upon cats which I made in April the heart was brought to a standstill and maintained at a standstill for periods of half a minute when the vagus was stimulated with a faradic current of moderate strength.

*After-effects of stimulation of one vagus.* After the cessation of a stimulation of the vagus during which the heart had been slowed or

<sup>1</sup> HOUGH; *op. cit.*, p. 164.

brought to a standstill, the beat usually returned quickly to its previous rate, and there was neither a secondary acceleration, as is often observed when the vagus of the dog has been stimulated, nor a long-continued slowing, such as occurs frequently when the vagus of the opossum<sup>1</sup> has been stimulated. The blood-pressure after stimulation of the vagus was, however, often very irregular; this irregularity was much more marked in the spring than in the autumn or winter months. These irregularities of the blood-pressure were frequently accompanied by irregularities of the heart-rate.

During the months of the spring, however, there sometimes occurred even before stimulation, and without any apparent cause, marked irregularities of the blood-pressure and the heart-rate, whereas during the autumn months one of the most striking characteristics of the tracing had been the great regularity of the blood-pressure and of the heart-rate; as a rule, the latter varied very little in the course of a long experiment, but continued almost the same till the animal was at the point of death. In fact, during the spring months there seemed to be a want of stability or of general tone on the part of the heart and perhaps of the vaso-motor system, a condition which seems to me to resemble that which I have observed in frogs in the spring, when studying the form of the muscle-curve with the pendulum-myograph: the muscle-curves being very irregular although the conditions of the experiments were kept as nearly constant as possible.

*Rise of blood-pressure following stimulation of the vagus.* At times, after the cessation of a stimulation of the vagus in the guinea-pig, the blood-pressure rose far above its original height (see Figs. 2 and 3); in one case it was doubled. The cause of such a marked rise of blood-pressure following inhibition deserves investigation. One explanation of this phenomenon which might be suggested is that the vagus nerve is the trophic nerve of the heart, that stimulation of this nerve causes constructive metabolism to be induced during the period of inhibition, and that the heart is thus "nursed into more vigorous activity." The "good effects" upon the heart of vagus inhibition artificially induced in the warm-blooded animals may, I think, be questioned. My experience with the guinea-pig would seem to support the view that caution should be used in drawing very detailed conclusions with regard to specific physiological conditions in one kind of animals from experimental work on animals very

<sup>1</sup> HUNT and HARRINGTON: *Journal of experimental medicine*, 1897, ii, p. 715.

different morphologically and, in general, physiologically. The infrequently beating hearts of the cold-blooded frog and turtle are very different in several respects from the frequently beating hearts of the warm-blooded rabbit and guinea-pig. The opinion that inhibition caused by vagus stimulation is beneficial to the heart is largely the

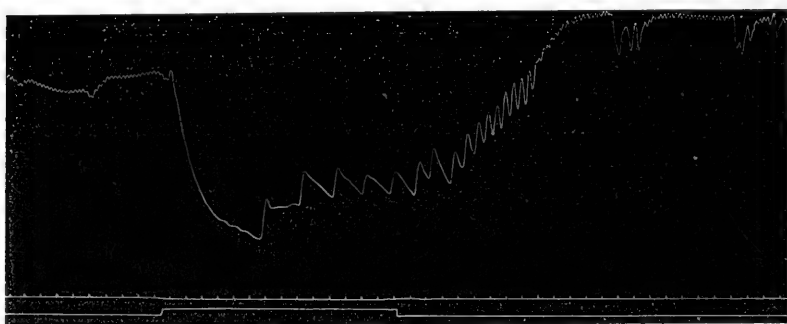


FIGURE 3. Three-fifths the original size. Inhibition from stimulation of the right vagus of the guinea-pig with the secondary coil at 15 cm. Time in seconds. The line of zero pressure is not shown. The writing-points of the manometer and of the stimulating key were in the same vertical line.

result of the teaching of Gaskell, but it must be remembered that Gaskell concludes his paper<sup>1</sup> by stating that his observations were confined to the cold-blooded animals, and that he is "not yet in position to say how far, in the more highly-developed mammalia, the phenomena of the heart may have become changed under a greater differentiation of function, and an increased complexity of structure." Immediate supply of nutriment is a matter of comparatively little importance to the cold-blooded hearts; it is a matter of the greatest importance to the very frequently beating warm-blooded hearts. "The two organs of the body whose supply of oxygenated blood cannot be interrupted, even temporarily, without danger to the system are the central nervous system and the heart."<sup>2</sup> "The force of the ventricular contraction is immediately affected by a change in the amount of blood supplied to the cardiac muscle,"<sup>3</sup> and "more important than the quantity is the quality of the blood flowing through

<sup>1</sup> GASKELL: *Journal of physiology*, 1883, iv, p. 124.

<sup>2</sup> ROY and ADAMI: *Philosophical transactions*, 1892, clxxxiii, B, p. 290.

<sup>3</sup> MAGRATH and KENNEDY: *Journal of experimental medicine*, 1897, ii, pp. 13, 14.

the coronary vessels.”<sup>1</sup> During any prolonged period of cardiac inhibition with a fall of blood-pressure in the aorta, the supply of nutriment to the heart muscle must certainly be diminished, and, as the right side of the heart is inhibited as well as the left, the arterIALIZATION of the blood must be diminished as well, and hence its quality deteriorated. So it seems very probable that any good effects upon the heart due to stimulation of trophic fibres may easily be counter-balanced by the injurious effects just pointed out. Hence a sudden and marked rise of pressure following inhibition is very probably due to some cause other than improvement in the nutrition of the heart. Moreover, this rise probably is not due to stimulation of the vaso-constrictor centre by a too venous condition of the blood; the rise occurs too suddenly to admit of such an explanation.

Whatever may prove to be the correct explanation of a rise of blood-pressure in some guinea-pigs after stimulation of the vagus, this rise is almost invariably followed immediately by a fall of blood-pressure below the normal, and very frequently by sudden failure of the heart and death of the animal. Fig. 3 is a tracing showing cardiac inhibition from stimulation of the vagus immediately after the animal had been prepared. After the cessation of the stimulation there took place a considerable rise of blood-pressure; the heart's beat became irregular, and the animal died within two minutes, death being preceded by a very rapid fall of blood-pressure. Throughout the whole series of my experiments stimulation of the vagi seemed to lessen the efficiency of the heart. This was especially true in the months from February to April, when the vitality of the animals was low, but it was also true in the months from October to January.

*“Escape” of the heart from vagus stimulation.* The heart of a guinea-pig when brought to a standstill by stimulation of the vagus soon begins to beat again; or, when slowed, to increase the frequency of its beats. The longest period during which I was able to maintain a standstill by stimulating the vagus was twenty-one seconds, and in this case death followed almost immediately, as was usually the case when the vagus nerve had been stimulated for any length of time in the spring. The following experiments illustrate the rate of escape during that season. I have no record of escape during the autumn, when the animals were in a more vigorous condition.

<sup>1</sup> FOSTER: Text-book of physiology, 6th ed., 1893, p. 367.

*Experiment I.* Guinea-pig; weight 550 grams; corrected mean blood-pressure 69 mm. of mercury; normal heart-rate 32 (all of the rates given are for periods of 10 seconds); peripheral end of right vagus stimulated while the secondary coil was at 15 cm. Rates during stimulation:  $6\frac{1}{2}$ , 9, 15, 16, 8, 6, death.

*Experiment II.* Guinea-pig; weight 637 grams; corrected mean blood-pressure 84 mm.; normal heart-rate 38; peripheral end of right vagus stimulated, secondary coil at 10 cm. Rates during stimulation: 3, 17, 15, 19, 25, 31, 35. The heart failed suddenly after the rate had increased to 35 and the blood-pressure had nearly reached the normal.

*Effect of shunting the current from one vagus nerve to the other.* It is an interesting question whether there be a single inhibitory mechanism in the heart or one for each vagus nerve. It is believed by many<sup>1</sup> that the same intracardiac apparatus serves for both nerves,—that after the heart of a mammal has begun to escape from inhibition produced by stimulation of one nerve, shunting the current into the other nerve does not cause a second standstill. I have found the contrary to be true in the guinea-pig, and have repeatedly caused a second standstill by suddenly shunting the current into the second nerve after escape had commenced from the inhibition caused by continued stimulation of the first nerve. This was tried, however, only in the spring months, when the cardiac-inhibitory mechanism was perhaps unusually irritable.

The following case has several features of interest:—

*February 22, 1897.* Guinea-pig; weight 510 grams. Chloral and morphine. Corrected mean blood-pressure 83 mm.; pulse-rate 37 in 10 seconds. Both vagi cut. Secondary coil at 12 cm. Stimulation of peripheral end of left vagus; heart stopped 10 seconds and blood-pressure fell to 26 mm.; heart's rate increased to 5 beats in 10 seconds and blood-pressure rose to 53 mm.; current shunted to right nerve; heart stopped 11 seconds and blood-pressure again fell to about 26 mm. The heart then rapidly increased in rate (the stimulation still continuing), gave a few irregular beats, and suddenly increased to 55 beats in 10 seconds, while the rate before stimulation had been only 37. Following a short period (12 seconds) of frequent beating, there occurred a period of infrequent irregular pulsations alternating with frequent beats. This state of things was allowed to continue for half a minute, when the current was shunted back into the left nerve; the heart was again stopped for four seconds. The heart's rate suddenly increased to 42 in 10 seconds,

<sup>1</sup> HÜFLER, E.: Die abgestufte Reizung des Herzvagus. *Archiv für Physiologie*, 1889, p. 295. HOUGH, *op. cit.*, p. 198.



when a short period of frequent and irregular beating was followed by failure of the heart and death of the animal, the stimulation having been maintained to the end. This is the only case in which acceleration was observed to occur during continued stimulation of the vagus. It is the only case in which acceleration was observed, except a few cases in which it appeared as an after-effect of inhibition.

*The comparative efficiency of the two vagi.* In a number of species of animals one vagus, usually the right, has been found to exert a more powerful influence upon the heart than does the other. In the guinea-pig I have found both vagi to be always efficient, and in no case have I been able to discover any greater efficiency on the part of one than on that of the other.

In quite a number of cases simultaneous stimulation of both vagi has not been observed to give more powerful inhibition than stimulation of the same strength applied to either nerve separately.

### III. THE DEPRESSOR NERVE.

There is found in the guinea-pig in close relation to the vagus and cervical sympathetic a very delicate nerve, stimulation of which causes a fall of blood-pressure resembling in every respect that caused by stimulating the depressor in the rabbit. This nerve can usually be found by raising the carotid artery out of its sheath on a curved

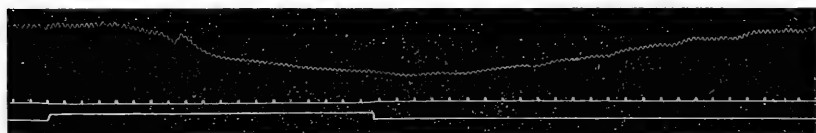


FIGURE 4. Three-fifths original size. Fall of blood-pressure as a result of stimulation of the central end of the left depressor with the secondary coil at 8 cm. Time in seconds. The line of zero pressure is not shown. The writing-points of the manometer and of the stimulating key were in the same vertical line.

needle and thus putting the tissues between the carotid and the vagus on the stretch, the nerve is then seen extending through the connective tissue as a delicate whitish filament. At least part of this nerve can be traced to the ganglion of the trunk of the vagus, and in so far its origin resembles that of the depressor in the opossum; <sup>1</sup> whether, as in the opossum, there is a second root springing from the superior laryngeal nerve I have not been able to determine satisfactorily.

<sup>1</sup> HUNT and HARRINGTON: *op. cit.*, p. 717.

Stimulation of the central end of this nerve gives results very similar to those observed when the depressor of the rabbit or of the opossum is stimulated, namely, a fall of blood-pressure (Fig. 4) and moderate slowing of the heart's rate if the vagi are intact.

A number of attempts were made to stimulate the various branches of the stellate ganglion to determine whether they contained cardiac accelerator nerves, but as this work was deferred until the spring, it was found that the animals did not possess sufficient vitality to permit the necessary dissection and prolonged experiment.

In conclusion I desire to express my indebtedness for valuable assistance and suggestions to Professor Frederic S. Lee and to Dr. Reid Hunt.

## PHLORHIZIN DIABETES IN DOGS.

BY F. H. REILLY, F. W. NOLAN, AND GRAHAM LUSK.

[From the *Physiological Laboratory of the Yale Medical School.*]

IN a paper published<sup>1</sup> recently from this laboratory it was shown that when starving rabbits are kept under the constant influence of phlorhizin<sup>2</sup> by frequent subcutaneous administration of the drug, a constant relation approximating the ratio 2.8:1 is established between urinary dextrose and nitrogen, after the preliminary sweeping out of the systemic sugars through the urine. This ratio is the same as that already discovered by Minkowski in total pancreas diabetes in dogs. From the meagre experiments of others which could be used for comparison, the conclusion was drawn in the article just cited that like results would be obtained by treating dogs in a manner similar to that employed for rabbits. Various experiments preliminary to the present research, however, did not give the results expected. The ratio in dogs was found always higher than in rabbits, as is seen in the following table: —

DOG I. Weight 24.1 kg. 3 grams Phlorhizin every 8 hours.

Date.	Dextrose.	Nitrogen.	D : N	Food.
Jan. 5, 1897	53.07	11.54	4.51	. . . .
6,	64.49	11.16	5.77	. . . .
7,	61.63	16.29	3.78	. . . .
8,	60.38	16.36	3.69	. . . .
9,	73.05	20.45	3.57	{ 30 grams meat per hour for 10 hours.

<sup>1</sup> LUSK, G., unter Mithilfe von E. L. MUNSON, E. A. LAWBAUGH, und I. M. HELLER: *Zeitschrift für Biologie*, 1898, xxxvi, p. 82. The general literature on the subject of phlorhizin diabetes is discussed in this article, and need not be re-stated here.

<sup>2</sup> This word is also spelled phloridzin and phlorizin, which are incorrect (Külz und Wright, *Zeitschrift für Biologie*, 1890, xxvii, p. 181). Webster and the *Century Dictionary* give phlorizin, but the more correct spelling implied by the Greek derivative *φλοιόρριζος* demands the use of phlorhizin.

On account of the above experiment we came to the conclusion that the excess of sugar was removed in the dog more gradually than in the rabbit, and we decided to continue the constant influence of phlorhizin over a considerable period of time, with a view to the ultimate establishment of a ratio  $D : N :: 2.8 : 1$ . In this and in all the following experiments the dogs fasted two and a half to three days before the first administration of phlorhizin. In the following experiment, on the fifth day of diabetes and the seventh of fasting, the dog was so weak that it could not stand up and so 100 grams of lean meat cut free from fat was given thereafter every eight hours. The dog's condition greatly improved, and remained satisfactory until nearly the end of the experiment. On the last day it was not so weak as the day before we began to feed meat.

Phlorhizin warmed in a 1.2 per cent solution of sodium carbonate was injected subcutaneously every eight hours. [See Table, Dog II.]

It will be seen that the ratio  $2.8 : 1$  was not to be obtained on the eleventh day of phlorhizin diabetes in dogs, but that the ratio remained constantly higher. The same drug together with analytical reagents yielded upon the second day of an experiment on a fasting rabbit the ratio  $2.89 : 1$ , and on the third day  $2.69 : 1$ , ratios which are characteristic in the rabbit. The average ratio found in the dog during the last nine days was  $3.75 : 1$ . No appreciable change in the ratio was brought about by feeding meat.

The total amount of dry fæces passed during the thirteen days was 67.35 grams, containing 3.02 grams of N or 4.20 per cent. This represents a daily amount of 0.23 grams of nitrogen. The fæces were pitch black, like hunger or meat fæces in dogs, and frequently here and in other experiments were accompanied by diarrhœa. This fact led sometimes to the mixing of urine and fæces, as may be noted in experiments still to be cited.

The sugar in the urine is dextrose, for it is completely fermentable with yeast, is only slightly affected by boiling with 8 per cent hydrochloric acid, and its specific rotation in the polariscope agrees with the reducing power determined according to the method of Allihn. Here then is a new level of sugar production from proteid. If we assume that 6.25 grams of proteid are burned for each gram of urinary nitrogen (neglecting that in the fæces), then 3.75 grams of dextrose may be derived from 6.25 grams of proteid, or approximately 60 per cent of dextrose from the molecule.

If instead of averaging the ratios of the last  $8\frac{1}{4}$  days we take the

DOG II.

Date.	Amount of urine in cc.	Weight in kg.	Dextrose.	Nitrogen.	D : N.	Phlorhizin every 8 hours.	Food every 8 hours.
Mar. 5, 1897	?	21.4	....	4.04	....	....	....
6,	107	....	....	4.17	....	....	....
7,	640	....	63.55	12.66	5.02	2.5 grams	....
8,	746	....	65.30	18.76	3.38	"	....
9,	960	20.1	65.84	18.57	3.54	"	....
10,	1075	....	64.80	17.29	3.74	"	....
11,	1425	18.6	77.48	21.45	3.61	5 grams	100 gr. meat
12,	1490	....	71.12	18.17	3.91	"	"
13,	1492	....	66.71	18.30	3.64	2.5 grams	"
14,	1670	....	69.04	17.63	3.91	"	"
15,	1510	17.6	62.01	16.06	3.80	"	"
16,	1215	....	57.70	15.80	3.65	"	{ 100 gr. meat + 25 gr. lard
17,	430 <sup>1</sup>	17.2	16.90	4.54	3.72	"	100 gr. meat.

<sup>1</sup> 6¼ hours' urine. Glycogen in the liver = 0.392 gr. = 0.08 per cent.

relation between the sum of the dextrose and nitrogen, we obtain 550.6 grams D : 147.8 grams N :: 3.72 : 1. By adding to this nitrogen that derived from the faeces ( $8\frac{1}{4} \times 0.23$ ) we obtain the following ratio : 550.6 grams D : 149.7 grams N :: 3.67 : 1. According to this latter ratio the amount of sugar obtainable from proteid may be 58.7 per cent of the molecule.

This great sugar excretion is accompanied by a highly remarkable rise in the proteid metabolism above that in fasting. Thus on the third day of fasting 4.17 grams of nitrogen were eliminated, whereas

on the fifth day of fasting and the second of diabetes the nitrogen in the urine amounted to 18.76 grams, an increase of 450 per cent. Another experiment shows an increase of 540 per cent and two more an increase of 340 per cent each. A parallel to this rise in proteid metabolism is only to be found in the literature of phosphorus poisoning, and indeed the cause of the high metabolism, namely, the non-burning of the carbohydrates, would seem to be identical in the two cases. In the case of diabetes the sugar is removed and its well-known sparing influence over proteid metabolism is eliminated.<sup>1</sup> The explanation of the similar metabolism in phosphorus poisoning which seems most plausible to us is that the proteid sugar instead of being burned is converted quantitatively into fat, with the resulting high proteid metabolism noted in fatty degeneration. Experimental evidence upon this part of the subject is being now obtained.

It will be noted above that there is no difference in the sugar excretion whether 2.5 grams or 5 grams of phlorhizin be administered every eight hours.

**Fat.**—Feeding 25 grams of lard does not increase the sugar excretion, a result which was to be expected from the work of Moritz and Prausnitz.<sup>2</sup>

**Behavior of Ingested Sugars.**—The discovery of this intense form of diabetes in dogs led to the question of the behavior of different sugars after they had been fed to such dogs. In the following experiment 100 grams of lean meat was fed every eight hours from the fourth day of diabetes, and the dog being very fat bore the experiment extremely well. The idea was to obtain D:N::3.75:1, and then to feed the various monosaccharides which enter into physiological consideration, namely dextrose, levulose, and galactose. The "extra sugar" in the urine of these dogs can be estimated by multiplying the nitrogen in the urine by 3.75 (which represents sugar from proteid) and subtracting this from the total sugar found for the day. Any such "extra sugar" would be derived from the sugar fed. The sugar dissolved in water was readily taken by the dogs and was fed in portions of eight grams every six hours, commencing immediately after the urine had been drawn off in the morning.

<sup>1</sup> LUSK: *loc. cit.*, p. 97; also LUSK: *Zeitschrift für Biologie*, 1890, xxvii, p. 459.

<sup>2</sup> MORITZ and PRAUSNITZ: *Zeitschrift für Biologie*, 1890, xxvii, p. 81.

DOG III. — 2 grams Phlorhizin every 6 hours after April 14.

Date.	Amount of urine in c.c.	Weight.	Dextrose.	D by polar.	Nitrogen.	D : N.	Extra sugar.	P <sub>2</sub> O <sub>5</sub> .	N : P <sub>2</sub> O <sub>5</sub> .	Food.
April 12, 1897	455	27.3	....	....	4.09	....	....	0.913	5.3	. . . . .
13,	288	....	....	....	2.79	....	....	0.485	5.7	. . . . .
14,	865	....	38.95	....	4.12	9.46	23.50	0.354	11.6	. . . . .
15,	1255	....	46.22	....	9.49	4.87	10.64	0.990	9.6	. . . . .
16,	1670	....	53.41	....	8.69	6.14	20.82	1.045	8.3	23.77 grams dextrose.
17,	1950	25.8	58.68	....	13.46	4.36	8.21	2.000	6.7	100 gr. meat every 8 hours.
18,	2000	....	50.29	....	12.90	3.89	....	2.185	5.9	" "
19,	2330	....	66.66	....	11.78	5.66	22.49	1.587	7.4	Meat + 24 gr. dextrose.
20,	1765	....	51.74	....	13.15	3.94	....	1.710	8.2	Meat.
21,	1872	25.0	63.27	57.5	12.79	4.95	15.31	1.531	8.3	Meat + 24.05 gr. levulose.
22,	1640	....	44.56	44.0	11.65	3.82	....	1.278	9.1	Meat.
23,	1885	....	51.85	49.4	11.85	4.37	7.42	1.352	8.8	Meat + 24.02 gr. levulose.
24,	1285	....	35.10	32.0	9.91	3.54	....	1.200	8.2	Meat.
25,	1820	24.9	51.59	50.6	11.65	4.43	7.89	1.650	7.0	Meat + 24 gr. galactose.
26,	1380	....	47.79	44.8	9.55	5.00	1.198	1.120	8.5	" "
27,	1355	....	41.61	39.1	10.39	4.00	....	1.292	8.0	Meat.
April 28, 1897/ 8 hours' urine)	445	24.3	11.87	10.5	3.37	3.53	....	0.498	6.7	Meat.

Average of last six meat days = D : N :: 3.79 : 1. Glycogen in liver = 0.936 grams = 0.31%. Glycogen in voluntary muscle = 0.37%.  
 April 19, 26, and 21, the dog received one gram phlorhizin every three hours, except at 1 and 7 A.M., when 2 grams were given, and 4 A.M., when none was given.

*Dextrose.* On the first day of dextrose feeding, 20.82 grams of extra sugar was present in the urine, following an ingestion of 23.77 grams of dextrose. The experiment was however inconclusive, since the fasting ratio between dextrose and nitrogen had not been obtained. The day before the second feeding of dextrose, the ratio between dextrose and nitrogen was 3.89:1; on the sugar day, 5.66:1; and on the following day 3.94:1. The extra sugar here was 22.49 grams following an ingestion of 24 grams of dextrose. A third experiment is taken from the unpublished work of Messrs. T. S. McDermott and W. E. Ray done in this laboratory and is given below.

DOG IV. — Weight 10 kg. 1 gram Phlorhizin every 8 hours.

Date.	Dextrose.	Nitrogen.	D : N.	Extra sugar.	Food.
Nov. 18, 1897	36.72	9.63	3.81	....	.....
19,	29.66	7.04	4.20	....	.....
20,	48.40	10.29	4.70	9.82	{ 150 grams meat + 11.76 grams dextrose.
21,	41.52	10.89	3.81	....	
					150 grams meat.

Here we have on the sugar day a ratio of 4.70:1 and an excretion of 9.82 grams of extra sugar following upon an ingestion of 11.76 grams.

We can conclude from our experiments that the dextrose fed is very nearly quantitatively eliminated. These experiments prove also that, within these limits at least, very little loss takes place through fermentation.

*Levulose.* After feeding 24.05 grams of levulose, 15.31 grams of extra sugar appeared in the urine. The extra sugar could not be destroyed by heating the urine for several hours at 100° with 8 per cent hydrochloric acid. It therefore was not levulose. The reading in the saccharimeter was lower than it should be. Two causes might have contributed to this: first, the lævo-rotary substances of phlorhizin urines discovered by Cremer;<sup>1</sup> second, personal error, it being the first time this particular saccharimeter was used, and the solution being so weak that a difference of 0.2 per cent would account for the discrepancy between 63.27 grams and 57.5 grams.

<sup>1</sup> CREMER: Zeitschrift für Biologie, 1898, xxxvi, p. 115.



On the second feeding of 24.02 grams of levulose, 7.42 grams of extra sugar appeared in the urine, and here the totals of dextrose by Allihn's method and by polarization were respectively 51.85 and 49.4 grams. After ingestion of levulose is seen therefore its well-known partial conversion into dextrose, which in phlorhizin diabetes is quantitatively eliminated. It is especially remarkable that the burning levulose does not affect the proteid metabolism. Possibly the levulose may be burned by the sugar-hungry cells of the villus as soon as it is absorbed, and therefore its influence on metabolism is not widespread, but limited to certain localities. Minkowski<sup>1</sup> has shown that levulose if fed to diabetic dogs may increase the glycogen in the liver. In virtue of the small amounts of levulose used in our experiments, it seems improbable that it could reach the liver before combustion, and we would suggest that possibly the epithelium of the villus likewise may have the power for this conversion.

*Galactose.* Results similar to those obtained after feeding levulose were found on feeding galactose. In both cases of galactose feeding the extra sugar consisted of dextrose. This was determined by the fact that the specific rotation of the solution indicated the same amount of dextrose as was shown by the method of Allihn. For dextrose  $(\alpha)_D = +52.6^\circ$ , for galactose,  $+83.8^\circ$ . Galactose may therefore in part be converted into dextrose.

*Phosphates.* — It has been stated by von Ackeren<sup>2</sup> that phosphates in diabetic urine are present above the amount normally pertaining to the proteid decomposition. The phosphates were therefore determined in the phlorhizin urine by titration with uranium nitrate. The results show that after fifteen days of diabetes there is no marked change from the usual ratio between phosphates excreted and proteid metabolism. This result is in confirmation of the experiments of Tenbaum<sup>3</sup> in diabetes mellitus, who also finds the calcium excretion to be unchanged.

*Glycogen.* — Phlorhizin diabetes fails to remove glycogen entirely from the liver and muscles. Glycogen in the liver of Dog II amounted to 0.392 grams = 0.08 per cent; in the liver of Dog III, 0.936 grams = 0.13 per cent, and in the muscle of the hind leg the large amount of 0.37 per cent was found. The dogs were killed while

<sup>1</sup> MINKOWSKI: Archiv für exper. Pathol. und Pharmakol., 1893, xxxi, p. 165.

<sup>2</sup> VON ACKEREN: See von NOORDEN: Pathologie des Stoffwechsels, 1893, p. 416.

<sup>3</sup> TENBAUM: Zeitschrift für Biologie, 1896, xxxiii, p. 379.

under the influence of morphine, which prevents loss of glycogen from struggling.

More frequent administration of phlorhizin, *i. e.*, once every three instead of once every six hours, does not change the ratio D : N in the urine.

**Sugar production from Meat and Gelatine.**—We have seen that feeding meat in small quantities during the day does not change the ratio D : N. Experiments were now tried with larger amounts of meat and with gelatine to seek for the ratio under these circumstances. On the days of meat feeding the urine was analyzed in fractions of equal periods, as will be described later. The general results for twenty-four hours urine are given below, two dogs having been experimented upon.

DOG V.—1 gram Phlorhizin every 8 hours after Feb. 22.

Date.	Amount of urine in cc.	Weight in kg.	Dextrose.	Nitrogen.	D : N.	Food.
Feb. 22, 1898	76	12.8	....	2.49	....	. . . .
23,	393	....	40.36	6.88	5.84	. . . .
24,	555	....	47.47	13.49	3.52	. . . .
25,	625	....	47.73	14.01	3.41	. . . .
26,	1013	....	74.95	21.11	3.55	500 grams meat.
27,	576	....	42.32	11.53	3.67	. . . .
28,	910	....	53.17	14.52	3.66	60 grams gelatine.
Mar. 1, 1898	495	....	34.20	9.21	3.71	. . . .
2,	790	....	63.95	18.39	3.42	500 grams meat.
Mar. 3, 1898/ 6 hours' urine)	84	10.6	6.93	1.68	4.12	. . . .
Average for last eight days: D : N :: 3.63 : 1. Rise in N excretion during starvation = 560 %.						

Before entering into the detailed discussion of the experiments, a few brief remarks on the theory of the method of proteid destruction within the organism would seem to be necessary. The well known theory of Voit<sup>1</sup> maintains that there is a preliminary cleavage of the

<sup>1</sup> VOIT: Hermann's Handbuch der Physiologie, 1881, vi, 1, p. 295.

DOG VI.—2 grams Phlorhizin every 8 hours after Feb. 6.

Date.	Amount of urine in cc.	Weight in kg.	Dextrose.	Nitrogen.	D : N.	Food.
Feb. 3, 1898	....	36.71	....	....	....	. . . .
6,	770	....	....	8.99	....	. . . .
7,	1730	....	69.36	13.49	5.14	. . . .
8,	2555	....	74.06	18.58	3.98	. . . .
9,	2895	....	69.72	18.04	3.86	. . . .
10,	3270	....	79.66	23.79	3.30	105 grams gelatine.
11,	2160	....	50.23	14.66	3.42	. . . .
12,	4181	....	85.94	27.49	3.13	870 grams meat.
13,	1970	....	52.11	12.44	4.19	. . . .
14,	2860 <sup>1</sup>	....	41.60	19.67	2.11	105 grams gelatine.
15,	2260	....	29.40	11.73	2.70	. . . .
16,	{ B 1528 <sup>2</sup> A 238 <sup>3</sup>	....	22.43	6.80	3.31	20 grams levulose.
		....	4.58	1.65	2.77	. . . .
17,	570 <sup>4</sup>	27.7	7.23	2.37	3.05	. . . .
<sup>1</sup> Albuminuria. <sup>3</sup> Some lost through mixture with fæces. <sup>2</sup> 16 hours' urine. <sup>4</sup> 6 hours' urine.						

proteid molecule in metabolism into a nitrogenous radicle, and into a non-nitrogenous radicle, each of which may subsequently be burned within the cells at different times. The experiments of Feder<sup>1</sup> have shown that after feeding meat to a starving dog the quantity of nitrogen in the urine rises, attaining a maximum at the sixth to the eighth hour, and then continuously sinks, so that at the fourteenth hour most of the nitrogen corresponding to the meat eaten has been eliminated. The excretion of CO<sub>2</sub> under these circumstances is, however, much more evenly distributed over the course of the twenty-four hours. Voit<sup>2</sup> concludes therefore that there is an early cleavage of the proteid molecule through which little energy is liberated; that there is a rapid combustion of the nitrogenous radicle, as shown by the elimination of the nitrogenous end products in the urine;

<sup>1</sup> FEDER: *Zeitschrift für Biologie*, 1881, xvii, p. 541.

<sup>2</sup> VOIT: *Zeitschrift für Biologie*, 1891, xxviii, p. 291.

and that the non-nitrogenous radicle which contains the major part of the potential energy of the molecule may in part be temporarily stored either as glycogen or as fat, and be fed to the tissues more evenly during the course of the twenty-four hours as need requires. Now the indications are that phlorhizin acts quickly, removing sugar from the organism as soon as it is produced. If Voit's non-nitrogenous portion of the proteid consist of dextrose and the idea of its quick cleavage from proteid be true, then on feeding meat to a phlorhizin dog more sugar should appear in the urine of the first hours after feeding than corresponds to the nitrogen eliminated.

The results of one experiment after feeding 500 grams of meat to a fasting diabetic dog, and collecting the urine first in two periods of three hours each, and then in one of six hours, are given below. The urine was, of course, obtained through a catheter and the bladder was carefully washed with water. The meat was eaten within one minute.

DOG V.—Urine of Feb. 26.

	Amount in cc.	Dextrose.	Nitrogen.	D : N.
Preceding 3 hours (estimated) . .	....	5.96	1.75	3.41
1st 3 hours . . . . .	137	12.43	2.52	4.92
2d 3 hours . . . . .	193	14.70	3.76	3.91
3d 3 hours . . . . .	385	11.23	3.85	2.92
4th 3 hours . . . . .		11.23	3.85	2.92
Following 3 hours (estimated) . .	....	6.34	1.78	3.56

We start here with the estimated decomposition during three hours of fasting. During three hours after feeding meat the ratio in the urine equals D:N::4.92:1, and in a subsequent period of three hours the ratio reads 3.91:1, whereas during the next six hours it sinks to 2.92:1. This however is followed by a period in which the original fasting quantity of dextrose and nitrogen with the original ratio are approximately attained. If we take the whole period of twelve hours when meat was fed and compare it with the foregoing and following periods of like duration we discover the following relations:—

	Dextrose.	Nitrogen.	D : N.
Fasting, 12 hours (estimated) . .	23.87	7.00	3.41
After meat feeding, 12 hours . .	49.59	14.00	3.54
Subsequent 12 hours . . . . .	25.36	7.11	3.56

Since the meat period of twelve hours was followed by a period entirely similar to the fasting period from which the start was made, it follows that the meat was absorbed and burned almost entirely within the first twelve hours after feeding. And this table shows that in the aggregate the ratios D:N in all three periods are the same.

The sugar of eaten proteid is therefore entirely eliminated in phlorhizin diabetes, but it may be eliminated before the nitrogen belonging to it. Hence it is probably one of the more immediate cleavage products of the proteid molecule in metabolism.

It is impossible at the present writing to state whether the sugar in the urine of the above periods of three hours represents the amount of sugar formed within the three hours, and immediately eliminated, or whether the sugar excretion represents the maximum limit of renal activity, and hence its comparatively even distribution over the hours named.

The ability to gradually eliminate the sugar formed was illustrated in two experiments similar to the above but somewhat less satisfactory in their results. One was a subsequent experiment four days later on the same dog, when the general tone of the animal was less favorable. That the absorption and destruction of the meat within twelve hours was not due to special accident in the first experiment is shown by the fact still to be discussed that the dog was able to repeat the performance with 60 grams of gelatine two days later. In the meat experiment on dog VI the animal was not very strong, and subsequent to the experiment the ratio fell to approximately 2.8:1, which as will be explained later probably indicates a decreased power of the kidneys. The conditions in the following two experiments were therefore distinctly less favorable for the quick removal of sugar through the kidney. That certain amounts of sugar may be protected from combustion in the organism is illustrated by the experiment on a fasting diabetic rabbit, whose urinary sugar was increased

after convulsions (which brought about a conversion of glycogen into sugar) this sugar being eliminated not immediately within the first hour, but gradually during six hours.<sup>1</sup>

The following results have been obtained from the urines of meat-fed dogs after dividing the urine into separate portions corresponding to two hours each. Dog V received 500 grams of meat, Dog VI 870 grams at one meal.

	Dog V.			Dog VI.		
	Urine of March 2.			Urine of Feb. 12.		
	D.	N.	D : N.	D.	N.	D : N.
Preceding 2 hours (estimated)	2.85	0.77	3.71	4.18	1.22	3.42
1st 2 hours . . . . .	5.82	0.78 <sup>1</sup>	7.49	9.26	1.92	4.82
2d 2 hours . . . . .	6.86	1.69	4.05	11.04	2.97	3.72
3d 2 hours . . . . .	7.39	2.29	3.18	4.69 <sup>2</sup>	1.82	2.58
4th 2 hours . . . . .	6.77	2.60	2.60	7.74	3.57	2.17
5th 2 hours . . . . .	7.03	2.28	3.08	11.37	3.50	2.17
6th 2 hours . . . . .	6.72	2.15	3.12	7.44	3.11	2.39
Following 2 hours (estimated)	3.89	0.98	3.95	5.72	1.60	3.25

<sup>1</sup> The urine of the first ten minutes was voided outside the cage and lost.  
<sup>2</sup> A part of the urine was lost through admixture with fæces.

The curve of nitrogen excretion is not essentially different from that obtained by Feder in normal fasting dogs after feeding meat. In the first two hours in both of the above experiments we find a large quantity of sugar in the urine showing the high ratios 7.49:1 and 4.82:1. The complete excretion of the sugar, however, extends over some little time, which is probably due to deficient power of the kidney to remove the sugar, as has been explained above.

<sup>1</sup> LUSK: *Zeitschrift für Biologie*, 1898, xxxvi, p. 82. Dextrose fed to rabbits in large excess is burned in phlorhizin diabetes. The protecting power is therefore limited. Whether a similar power to burn carbohydrates when fed in excess is present in total phlorhizin diabetes in dogs is at present unknown to us.

For further clearness the following summary is added: —

	Dog V.			Dog VI.		
	D.	N.	D : N.	D.	N.	D : N.
Fasting 12 hours . . . .	17.10	4.60	3.71	25.11	7.33	3.42
Meat 12 hours . . . . .	40.59	12.49	3.25	51.54	16.89	3.04
Following 12 hours . . .	23.36	5.90	4.12	34.40	10.60	3.25
Following 6 hours . . . .	6.93	1.68	3.95	....	....	....
Following 24 hours . . .	....	....	....	52.11	12.44	4.19
Total subsequent to meat .	70.88	20.07	3.53	138.05	39.93	3.45

These experiments, especially the first one made on Dog V, give a full confirmation of Voit's views. There is a quick preliminary cleavage of proteid into sugar and an unknown nitrogenous radicle. The sugar amounts to about 60 per cent of the proteid molecule, and contains not far from 60 per cent of its physiologically available energy. Through the action of phlorhizin the dextrose may be rapidly removed, whereas normally it is a substance which in excess may be stored as glycogen, or may even contribute to the formation of fat, both of which substances may be subsequently burned in the cells as need requires.

**Absence of Putrefaction.**—The fact that, generally speaking, in the cases of phlorhizin diabetes in dogs, there is no appreciable change in the ratio D : N, whether meat be fed or whether the dog be fasting, would indicate complete absence of putrefaction, for it is hardly possible that sugar could be produced within the organism from leucin, tyrosin, and other amido bodies formed in putrefaction.

**Exceptional Cases.**—We have remarked in the case of Dog VI the decrease in the ratio D : N to the basis found in rabbits of 2.8 : 1. This was accompanied by albuminuria. Only one other similar case has come to our notice, and that is taken from the work of Messrs. McDermott and Ray, done in this laboratory upon a fasting dog. The dog weighed 8.5 kg., and like the former dog had albumin in the urine.

The albuminuria suggests renal disease, but why, in the apparent absence of any chemical difference, one fraction of the sugar in pro-

teid should behave differently from the other fraction, remains a mystery. This peculiarity seems to bring added proof of the at least partially renal character of phlorhizin diabetes noted by Zuntz.<sup>1</sup> Phlorhizin diabetes cannot be wholly renal in character, for some unknown influence must protect sugar from combustion.

DOG VII.—1 gram Phlorhizin every eight hours after Dec. 19.

		Dextrose.	Nitrogen.	D : N.
Dec. 19, 1897	. . . . .	....	3.23	....
20,	. . . . .	29.51	7.34	4.02
21,	18 hours . . . . .	28.34	10.36	2.73
	8 hours . . . . .	8.15	2.84	2.86
22,	16 hours . . . . .	15.64	5.39	2.90

**Gelatine.**— It has already been shown in work on the rabbit<sup>2</sup> that both nitrogen and sugar rise in the urine after feeding gelatine, and that nearly the proteid ratio is maintained between the two. More exact determinations have been possible with dogs, and among several attempted by us may be mentioned the two following. In the first case 105 grams of French gelatine (containing 14.91 grams N), and in the second case 60 grams (8.52 grams N) were fed. The gelatine was stirred into hot water and on cooling was broken in fragments and moistened with water containing 0.5 — 0.3 grams meat extract (= 0.05 — 0.03 grams N). The mass was eaten greedily. In the first experiment the day commenced at 8.45 A. M., and at 9.15 A. M. about one quarter of the gelatine was eaten, at 3 P. M. about one half, and the remainder at 4.55 P. M. In the second experiment the whole mass was eaten at one time immediately at the commencement of the day.

Both of these experiments show an increased elimination of sugar and nitrogen after feeding gelatine. In the first experiment the ratio D : N fell from 3.86 in fasting to 3.30 on the gelatine day and rose to 3.42 on the following fasting day. The results in the second experiment are more convincing because here the ratio hardly changes in the two fasting periods, and it will be noticed that it also remains

<sup>1</sup> ZUNTZ: Archiv für Physiologie, 1895, p. 570.

<sup>2</sup> LUSK: *loc. cit.*



DOG VI.	Dextrose.	Nitrogen.	D : N.	Food.
Feb. 9, 1898 . . . . .	69.72	18.04	3.86	. . . . .
10, . . . . .	79.66	23.78	3.30	105 grams gelatine.
11, . . . . .	50.23	14.66	3.42	. . . . .
DOG V.	Dextrose.	Nitrogen.	D : N.	Food.
Feb. 27, 1898, 12 hours (estimated)	21.16	5.76	3.67	. . . . .
28, { 12 hours . . . . .	35.85	9.70	3.69	60 grams gelatine.
{ 12 hours . . . . .	17.32	4.82	3.59	. . . . .
Mar. 1, 1898, 12 hours (estimated)	17.10	4.60	3.71	. . . . .

unaltered during the intervening period when gelatine was fed. We may therefore conclude that the same large percentage of dextrose is obtainable from the metabolism of gelatine as from proteid. In this second experiment the whole of the gelatine was apparently absorbed and destroyed within twelve hours after feeding, because the amount of nitrogen is similar in each of the fasting periods. A very considerable amount of proteid was evidently spared from destruction by the burning gelatine. The twelve hours urine after gelatine feeding shows the presence of 9.7 grams of N. The gelatine contained 8.4 grams N, and if 8 grams of this appeared in the urine, then an amount of proteid corresponding to only 1.7 grams N would have been burned during the twelve hours against 5.76 grams N found for the twelve hours preceding. This would indicate a sparing of proteid to the extent of 69 per cent by the gelatine. The considerable sparing power of burning gelatine over proteid is well known.

**Theoretical Considerations as to the Constitution of Proteid.**—The discovery that the proteid molecule yields on cleavage in metabolism an amount of sugar equal to 58.7 per cent (as near as we have been able to determine) leaves on the other hand a nitrogen-containing radicle in which the carbon and nitrogen would appear in the atomic ratio of 2.2 of C to 1 of N. It is evident from this that the nitrogenous radicle cannot in metabolism yield much leucin or tyrosin, which require much carbon, whereas glycocoll and the sulphur-con-

taining taurin are theoretically possible. It will further be remembered that modern theory regarding the synthesis of proteid in plants, supposes the formation of proteid from sugar through union with asparagin and glutamin. Asparagin contains 2 of C, glutamin  $2\frac{1}{2}$  of C to 1 of N. It is also impossible to ignore the fact that the carbohydrate portion may be even larger than 58.7 per cent, and may possibly contain the radicles of levulose, of galactose, or even of pentoses, substances which may in part escape elimination in phlorhizin diabetes. Again, there is evidence that the glucoside phlorhizin which yields dextrose in the laboratory is destroyed in the body. Hence the sugar of all compounds of dextrose, even in phlorhizin diabetes, may not appear in the urine.

#### SUMMARY.

1. Frequent subcutaneous injections of phlorhizin in fasting dogs establish ultimately the ratio in the urine of Dextrose: Nitrogen:: 3.75: 1, which indicates a production of 60 grams of dextrose from 100 grams of proteid. Taking the faecal nitrogen into consideration, the amount of dextrose obtained from proteid may be more accurately estimated at 58.7 per cent.

2. The proteid metabolism may increase above that in simple fasting to an extent as high even as 560 per cent.

3. Dextrose fed in phlorhizin diabetes is quantitatively eliminated. Levulose and galactose are not eliminated as such, but only in so far as they are converted into dextrose.

4. Feeding fat does not affect the ratio.

5. Feeding meat does not affect the ratio for the day, but the sugar from eaten proteid may be eliminated before the nitrogen belonging to it, on account of an early preliminary cleavage of the molecule.

6. Gelatine yields the same amount of sugar as proteid does. Gelatine spares much proteid from metabolism.

7. Intestinal putrefaction and fermentation can only slightly have affected the proteid or dextrose which were fed in our experiments.

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ON INTESTINAL ABSORPTION AND THE SALINE  
CATHARTICS.<sup>1</sup>

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IN his well-known paper on the absorption of salt solutions from the intestine Heidenhain<sup>2</sup> came to the conclusion that two distinct factors were involved in the process, — the osmotic pressure of the solution, and the “physiological activity” of the epithelium. The latter induces a constant current from the lumen of the bowel towards the blood vessels.

Hamburger<sup>3</sup> accepts Heidenhain’s account of the osmotic action, but attempts to show that his “physiological activity” is really a combination of the effects of certain physical forces. These are molecular imbibition; the intra-intestinal pressure, which induces filtration; and the suction induced by the blood current through the intestinal vessels, similar to that observed with the ordinary suction pump of the laboratory. Hamburger’s explanation of the intestinal absorption therefore involves an obscure process — the molecular imbibition — but differs from Heidenhain’s in not involving the living cell.

Heidenhain performed a few experiments with solutions of the saline cathartic magnesium sulphate, from which he found that the water was much more slowly absorbed than from corresponding solutions of sodium chloride. He explains this retarded absorption by supposing that the sulphate lessens the “physiological activity” and that movement of its solutions is therefore controlled more by

<sup>1</sup> Received by the editors May 5, 1898.

<sup>2</sup> HEIDENHAIN: Arch. f. d. ges. Physiol., 1894, lvi, p. 579.

<sup>3</sup> HAMBURGER: Archiv für Physiologie, 1896, p. 428.

their osmotic pressure than is the case when solutions of non-purgative substances such as common salt are employed. In some experiments in which sodium sulphate or sodium fluoride was added to solutions of sodium chloride the absorption was also retarded for the same reason, each of these salts weakening the "physiological activity," although the fluoride is much more powerful than the sulphate.

Of the factors involved in Hamburger's scheme, only one — the molecular imbibition — can be affected by a change in the salt contained in the solution, for the filtration and the suction of the blood current remain unchanged. Hamburger made a few experiments to satisfy himself that molecular imbibition could occur in dead tissues, but a much more extensive investigation of the subject had been made earlier by Hofmeister.<sup>1</sup> From Hofmeister's results it would appear that colloid substances (gelatine), and pieces of dried tissue, such as the wall of the bladder, are by no means indifferent to the solutions in which they are soaked. Thus much more of a solution of sodium chloride, and of the salt itself, was imbibed than of solutions of some other salts including some of the saline cathartics.

The more recent work of Hedin<sup>2</sup> to which we shall return later, indicates that the red blood cells also imbibe more freely the solutions of certain salts than those of others for which they seem to have less affinity.

We felt that some light might be thrown on the process of intestinal absorption by a more accurate definition of the group of saline cathartics, and we have for this purpose compared the rate of absorption from the intestine of a large number of salt solutions with that of a one per cent sodium chloride solution. On looking over the list of salts which are generally regarded as saline cathartics it is apparent that the anion, or acid constituent of the salt, is generally the determining factor. Thus sodium sulphate, potassium sulphate, sodium phosphate, potassium tartrate, potassium-sodium tartrate, potassium citrate, sodium citrate, potassium ferrocyanide, and sodium ferrocyanide, are all looked upon as cathartics, while sodium chloride, potassium chloride, sodium acetate, potassium acetate, etc., are believed to be indifferent so far as action on the bowel is concerned. It is evident therefore that the anion or acid constituent is the deter-

<sup>1</sup> HOFMEISTER: *Archiv für exper. Pathol. und Pharmakol.*, 1890, xxvii, p. 395; 1891, xxviii, p. 210.

<sup>2</sup> HEDIN: *Arch. f. d. ges. Physiol.*, 1898, lxx, p. 525.

mining factor here, for the cations K and Na occur in both groups. As regards the magnesium salts the basic constituent or cation would also seem to be involved, for while in magnesium sulphate and magnesium citrate the purgative anion might explain the effects, these salts are generally believed to be more active than the corresponding salts of the alkalies, and in addition magnesium chloride, magnesium oxide, and magnesium carbonate have also some cathartic action; the presumption is therefore strong that the Mg ion is not indifferent as the K and Na ions are.

We took up first the question of the purgative anions by comparing the rate of absorption of a large number of salts of soda. A preliminary note of our results was published in the Journal of the Boston society of medical sciences, January 18, 1898. Almost simultaneously, Höber<sup>1</sup> published an account of his investigations on the absorption of a number of salts, dwelling particularly upon the effect of the basic constituent. We have therefore confined our examination of the cations to a few experiments which were carried out mainly to confirm his results, which we have much pleasure in doing.

In order to compare the influence of the salts on the unknown factor in absorption, whether this be physiological activity or molecular imbibition, it is of course necessary to eliminate the influence of the known factor, osmosis, by using solutions of the same osmotic pressure. This may be most easily accomplished by forming solutions which cause an equal depression of the freezing point. Our point of departure was a solution of about one per cent sodium chloride, which gave a depression of the freezing point ( $\Delta$ ) of  $0.59 - 0.64^{\circ}$  C., estimated by Beckmann's apparatus. The other salts employed were dissolved in water, the freezing point of each solution determined, and more water or salt added until  $\Delta$  approached that of the sodium chloride solution. We are therefore unable to state the percentage composition of most of our solutions, but append the depression of the freezing point to each one (see protocols, page 429). The variation in the  $\Delta$  of the solutions may at first sight seem to be considerable, but the osmotic pressure never varied more than five per cent above or below the average; and when the solution of a salt showed any marked departure from the rate of absorption of the standard sodium chloride solution, care was taken to reduce the error arising from this variation in the osmotic pressure to a minimum. As a matter of fact a considerable variation in the osmotic pressure may

<sup>1</sup> HÖBER: Arch. f. d. ges. Physiol., 1898, lxx, p. 624, issued March 3.

be allowed without causing any appreciable difference in the rate of absorption, owing to the unavoidable errors of the method.<sup>1</sup> Thus a solution of NaCl  $\Delta$  0.67 was found to disappear as rapidly as one of  $\Delta$  0.61.

In our first experiments we used rabbits, but we could obtain no satisfactory results, as the absorption from the intestine seems to be extremely irregular in these animals. This may be partly due to the fact that it is impossible to empty the stomach and bowel by fasting of reasonable duration, and partly perhaps to the rabbit's intestine being more sensitive to handling than that of the other animals used. The cat's intestine gave somewhat better results, but here also the absorption was often irregular. The great majority of our experiments were performed on dogs, and we have left those on the rabbit entirely out of account, while the results obtained from the cat's intestine have always been controlled by others on the dog.

The dogs and cats were anæsthetized by the subcutaneous injection of morphine, followed by the administration of chloroform acetone by the mouth. In some cases ether or chloroform was given by inhalation, instead of chloroform acetone. In every case the animal fasted for 36-48 hours before the operation, and in our later experiments we obtained the best results from animals which had fasted for three or four days. This preliminary fasting appears to be of great importance in any experiment in which a regular absorption is necessary.

In all our experiments two or more intestinal loops were used. They were ligatured off in the usual way. We are quite aware of the objections to this method, for Hay<sup>2</sup> showed that ligation is liable to cause irritation of the bowel. Owing to the limited supply of dogs, however, we could choose only between using several short loops, or using one long loop a number of times, and we soon found that the necessary manipulations rendered a loop very unreliable after three or four injections. We have attempted to avoid the disadvantages of our method by using a series of controls. Thus, the experiment was commenced by ascertaining the rate of absorption of the standard solution in all the loops, and this control was repeated whenever any solution was found to deviate considerably from the

<sup>1</sup> In Experiments 21 and 23, solutions of salts which differed considerably in their osmotic pressure were used for a purpose apart from the general scope of the work.

<sup>2</sup> HAY: Saline cathartics, Edinburgh, 1884.

standard solution in rate of absorption. In this way any abnormality developed in an individual loop could be recognized. In addition one loop was injected each time with the standard solution, and we could thus eliminate any errors due to the general condition of the animal, and also those arising from the manipulation, for the control loop was treated in exactly the same manner as the others. In estimating the rate of absorption of any solution we have taken into consideration not only the variation in the particular loop in which it was contained, but also any change in the control loop. Our loops were much shorter than those of most other investigators, varying from 25 to 45 cm., but it seems to us that the unabsorbed fluid can be much more completely removed from these short loops than from the longer ones, and that one source of error may thus be lessened. On the other hand, the short loops have the disadvantage that greater irritation is produced by the close proximity of the ligatures; but we believe that our system of controls has reduced very greatly the error arising from this source as well as the error due to the different rate of absorption in different parts of the bowel (Heidenhain). A glass cannula was passed into each loop, fixed by a ligature, and closed by a short indiarubber tube and clamp. In some of the early experiments the loops were washed out before and after each injection of salt solution, but we found that the additional manipulation caused a larger error than that arising from the use of unwashed loops, and this procedure was abandoned in our later work. The loops were emptied by gently stripping them, and were not exposed to the air longer than was absolutely necessary in order to empty them completely.

The salts examined fall naturally into groups as follows:

1. *The halogen salts:* NaCl, NaBr, NaI, NaFl. Exp. 17, 18, 19. Of these salts the bromide seemed to be absorbed as rapidly as the chloride, while the iodide was sometimes absorbed more slowly. In Experiment 17, however, all three salts disappeared equally from the loops, and in this experiment it is noted that a fresh iodide solution prepared that morning was used. It seems possible that the divergence in Experiments 18 and 19 was due to the use of an older solution, in which some decomposition had occurred. Höber found the chloride the most easily absorbed of the three, then the bromide, and last the iodide. In our experiments we did not observe such marked differences in the rate of absorption as appear in his protocols, and in fact are inclined to hold that very little difference exists between these salts in regard to the rate of absorption of their solutions. The

fluoride, on the other hand, is absorbed with great difficulty, as is shown in Experiment 19 and in others not included in the published protocols. It always caused more or less congestion and inflammation of the loop, as is evidenced by the absence of absorption of the standard solution from the loop afterwards. Heidenhain also found that the presence of even a small percentage of fluoride in a salt solution retarded its absorption. The chloride, bromide, and iodide may thus be classed among the indifferent salts, while the fluoride has a very distinctly retarding effect on absorption.

2. *Other inorganic salts* :  $\text{Na}_2\text{SO}_4$ ,  $\text{Na}(\text{C}_2\text{H}_5)_2\text{SO}_4$ ,  $\text{NaNO}_3$ ,  $\text{Na}_2\text{HPO}_4$ ,  $\text{NaH}_2\text{PO}_4$ ,  $\text{MgSO}_4$ ,  $\text{KNO}_3$ ,  $\text{K}(\text{C}_2\text{H}_5)_2\text{SO}_4$ ,<sup>1</sup>  $(\text{NH}_4)_2\text{SO}_4$ . Experiments 1, 2, 9, 10, 12, 17, 18, 20, 21, 22, 23, 24. The simple sulphates were absorbed much more slowly than the chlorides, as has been observed by a number of workers on the subject. No evidence of inflammatory reaction was observed, and the loop rapidly returned to its normal condition when the solution was removed. Sodium ethyl-sulphate has been used occasionally as a mild saline purge, and from our experiments it would seem to stand midway between sodium chloride and sodium sulphate. No simple sulphate was contained in the fluid injected or in the residue. The nitrate solutions are more slowly absorbed than the chlorides, but much more rapidly than the sulphates, as was observed also by Höber. The use of the nitrates was followed in one of our experiments (Exp. 18) by very distinct signs of irritation and inflammatory reaction, the residue being blood-stained and containing large quantities of mucus. The nitrates are generally looked upon as being more irritant to the alimentary canal than such salts as the chlorides and sulphates, and although in the second experiment (Exp. 24) no signs of irritation of the bowel were present, we think it questionable whether the fluid found at the end of the experiment was really due to the lack of absorption or to an effusion into the bowel. The two phosphates proved almost identical in the rate of absorption, and may best be classed with sodium sulphate.

3. *Ferrocyanides and ferricyanides*. Experiments 21, 22, 23. These two salts seem to be absorbed as slowly as the sulphates. The ferricyanide solution contained no ferrocyanide when injected, but the residue gave a copious precipitate of Prussian blue, indicating that much of the ferricyanide had been reduced to the ferrocyanide. The effect is therefore probably due to the latter salt.

<sup>1</sup> This salt was found to contain a considerable quantity of ordinary sulphate.



4. *Salts of the fatty acids*: sodium formate, acetate, propionate, butyrate, valerate, caproate, cœnanthylate, and caprylate. Experiments 4, 7, 11, 13, 14, 15, 16, 19. The first six of these salts are absorbed as rapidly as the chloride. The cœnanthylate disappears somewhat more slowly, although in Experiment 15 this is not the case. The caprylate is somewhat more rapidly absorbed than the sulphate.

The lactate (Experiments 11 and 13) seems to lie midway between the chloride and sulphate of soda.

5. *Oxalic acid series*: Oxalate, malonate, and succinate of sodium. Experiments 3, 7, 8, 13, 25. The oxalate is but little absorbed, and always induces congestion and inflammation, from which the loop does not soon recover. It therefore resembles the fluoride. The malonate and succinate solutions are scarcely absorbed, but do not cause inflammation, and the loop recovers after the solution is removed.

6. *Tartrate, citrate, and malate of sodium*: Experiments 3, 5, 6, 7, 8, 13, 14. The solutions of these three salts are absorbed at about the same rate as those of the sulphates. The first two are well-known saline cathartics.

7. *Salts of the aromatic acids*: Salicylate, ortho-phthalate, and paraphthalate of soda. Experiments 12, 17. The phthalates seem to disappear more slowly than the chlorides, but do not retard absorption to the same extent as do the sulphates. The salicylate was also slowly absorbed, but was used in only one experiment, which is insufficient to determine its exact position.

8. *The metallic ions*: Sodium, potassium, ammonium, magnesium, barium, calcium. In all the experiments except 23, 24, and 25 these are combined with  $-\text{SO}_4$ ,  $-\text{NO}_3$ ,  $-\text{FeCy}_3$ , and  $-\text{FeCy}_4$  ions. In Experiments 23, 24, and 25, their chlorides and calcium acetate are compared. No difference could be detected in the behavior of the potassium and sodium salts. The ammonium chloride solution disappeared in Experiment 25 more rapidly than the standard sodium chloride solution, while in Experiment 24 it was absorbed at least as quickly; but here the sodium chloride solution was also entirely absorbed, so that it is impossible to state which was taken up the more rapidly from the bowel in this experiment. The salts of the alkaline earths were absorbed much more slowly than the corresponding salts of the alkalis. As regards the cations, therefore, our results are practically identical with those of Höber.

The soda salts can thus be arranged into four fairly distinct groups, according to the rate of absorption of their solutions.

TABLE I.

I.	II.	III.	IV.
Chloride. Bromide. Iodide.			Fluoride.
	Ethyl-Sulphate. Nitrate.	Sulphate. Phosphates.	
		Ferrocyanide. Ferricyanide.	
Formate. Acetate. Propionate. Butyrate. Valerate. Caproate.	CEnanthylate. Lactate.	Caprylate.	
		Malonate. Succinate.	Oxalate.
		Tartrate. Citrate. Malate.	
	Salicylate. Phthalates.		

The solutions of the salts of column I are all absorbed equally rapidly. Those of column II vary more or less in their behavior, but are generally absorbed more slowly than those of I. Those of III disappear very slowly, but, as a general rule, do not impair the absorption of the loop permanently, while the solutions of IV scarcely lessen at all in amount and evidently injure the loop seriously, for the solutions subsequently injected are only slowly absorbed or may even

increase in amount. We are not inclined to look upon this as differentiating the salts of IV from those of III qualitatively but only quantitatively, for the subsequent absorption was sometimes impaired by the salts of III, and Hay also found that strong solutions of sodium sulphate reduced the absorption of sodium chloride afterwards.

This table resembles in many features that given by Hofmeister to indicate the relative power of different salts to precipitate egg albumin.<sup>1</sup> His tables may be abbreviated by arranging the salts in two columns. It must be premised that the cations seem to have more influence here than in the intestine.

<i>Ions with little or no power of precipitation.</i>	<i>Ions with greater power of precipitation.</i>
Chlorides.	Sulphates.
Bromides.	Phosphates.
Iodides.	Acetates.
Nitrates.	Citrates.
Some acetates and chromates.	Tartrates.
	Chromates.

In experiments on the precipitation of gelatine with neutral salts he obtained similar results, and also in those on the precipitation of colloid iron oxide, while the sodium oleate solutions behave differently in regard to some salts.<sup>2</sup>

Hofmeister<sup>3</sup> found that gelatine plates absorbed less fluid when soaked in sulphate, tartrate, citrate, or acetate solutions than in chlorides, chlorates, nitrates, or bromides. Here, as in his experiments on the precipitation of egg albumin, the results are calculated for normal solutions so that they may be considered as isotonic except in so far as the dissociation of the salt is concerned. His experiments on the imbibition by pieces of the bladder wall were unfortunately carried out with percentage solutions and cannot be utilized for comparison. The same is true of Limbeck's<sup>4</sup> experiments on the diuretic action of salts.

It will be seen that Hofmeister's results do not quite correspond with ours, although they bear a very close resemblance. The most

<sup>1</sup> HOFMEISTER: *Archiv für exper. Pathol. und Pharmakol.*, 1888, xxiv, p. 247.

<sup>2</sup> HOFMEISTER: *Ibid.*, 1888, xxv, p. 1.

<sup>3</sup> HOFMEISTER: *Ibid.*, 1891, xxviii, p. 210.

<sup>4</sup> LIMBECK: *Archiv für exper. Pathol. und Pharmakol.*, 1888, xxv, p. 69.

striking difference is in the behavior of some of the acetates, which precipitate proteids and other colloids and prevent the imbibition of gelatine plates much more than the chlorides do, while in our experiments solutions of the acetates and chlorides are equally rapidly absorbed from the intestine.

In spite of this and of some other minor differences which may be found by comparing Hofmeister's original tables with ours, there is a very striking similarity in our results, — most of those salts which precipitate egg albumin and prevent the permeation of gelatine plates also retard the absorption of fluid from the intestine. This would seem to support the view that these salts act as saline cathartics not through their lessening the "physiological activity" of the intestinal wall, as Heidenhain supposed, but through their being devoid of some general relation to colloid substances, organized or unorganized. This would not entail the belief that absorption from the intestine is a purely physical process, for the suggested explanation only covers the absorption of the fluid into the epithelium, and does not attempt to account for its transmission to the bloodvessels.

On the other hand some facts point to the opposite conclusion, namely, that the reaction of the intestinal epithelium to the salts is not due to the general physical properties of colloids. Thus Hedin<sup>1</sup> investigated the behavior of the red cells of the blood in solutions of various ammonium salts, and found that they were permeated without resistance by the chloride, bromide, sulpho-cyanate, oxalate, ferro- and ferricyanide, lactate, and ethyl-sulphate, while the sulphate, phosphate, tartrate, and succinate penetrated them with difficulty. These results present much greater contrasts to ours than Hofmeister's do, for while the ions that penetrate the red blood cells with difficulty also prevent the absorption of fluids by the intestinal wall, several ions that permeate the blood corpuscles with ease act as cathartics (oxalates, ferrocyanides), and others stand midway between the cathartics and the indifferent ions (ethyl-sulphate, lactate). It is evident therefore that the colloids of the red blood cells and those of the intestinal epithelium differ very considerably in their relations to different anions, although there are some common features. This conclusion is confirmed by the fact that the red cells are permeated only with the greatest difficulty by the fixed alkali ions, whereas comparatively little resistance is offered by the intestine.

<sup>1</sup> HEDIN: *loc. cit.*

Again, Leathes and Starling<sup>1</sup> found that the pleural endothelium absorbed solutions of magnesium sulphate and sodium sulphate as rapidly as those of sodium chloride, so that here the cell contents present yet another variation in their affinities.

Lastly Pohl,<sup>2</sup> Young,<sup>3</sup> and others have investigated the precipitation of colloid carbohydrates by neutral salts and find a considerable variation in their relations. Pohl states that the sulphate of ammonia precipitates a larger number of these than the phosphate of ammonia or the acetate of potash, while these again act on a larger number than the sulphate of magnesium. The conclusion seems inevitable that while a general resemblance may exist in the relation of the neutral salts to the different groups of colloid bodies, the details vary with each individual colloid. This differentiation of salts into two series,—the one permeating the intestinal epithelium, the other apparently repelled by it, naturally demands explanation, and we have therefore attempted to find some further characters common to the cathartic salts and not possessed by the indifferent salts.

Loeb<sup>4</sup> has recently advanced the view that the action of some substances may be determined by the number of dissociated ions, and by their velocity. The amount of dissociation can scarcely be expected to have much importance, however, where identical effects are obtained with two salts which vary so greatly in their dissociation as the chloride and acetate of sodium. Dr. K. Guthe of the physical laboratory had the kindness to ascertain the relative electrolytic conductivity of our solutions, and we found that it bore no relation to their behavior in the intestine. For example, the sodium chloride solution gave a deviation of the electrometer of 158, the acetate of 97.5, the fluoride of 110, the oxalate of 155, the tartrate of 137, and the sulphate of 235. The purgative fluoride and oxalate therefore stand between the indifferent acetate and chloride. The variation in the velocity of the anions is also apparently without significance, for as it decreases with an increase in the atomic weight the purgative caprylate ion must have a smaller velocity than the indifferent caproate or acetate, while the oxalate ion on the other hand must have a greater velocity than the succinate, which however is less purgative.

<sup>1</sup> LEATHES and STARLING : *Journal of physiology*, 1895, xviii, p. 106.

<sup>2</sup> POHL : *Zeitschr. f. physiol. Chemie*, 1890, xiv, p. 151.

<sup>3</sup> YOUNG : *Journal of physiology*, 1897, xxi, p. xvi.

<sup>4</sup> LOEB : *Arch. f. d. ges. Physiol.*, 1897, lxix, p. 1.

The physical differences of the solutions do not present any relation to the differences in their action, then, and we have sought for some pharmacological property common to the cathartics, and not possessed by the indifferent salts. As regards the oxalate and fluoride (4th column, table I), this might be found in their action as general protoplasmic poisons; the connection between this and their action on the bowel is rendered more plausible by the fact that the addition of quinine hydrochlorate, a well known protoplasmic poison, to the standard solution prevents absorption and causes congestion and irritation, although the quantity added is too small to alter the osmotic pressure.

It is more difficult to find any relation between the substances of the third column, for while the tartrate and citrate are undoubtedly poisonous when injected into the blood, the sulphate has little or no such effect. Most of these salts are dibasic or tribasic, while those of the first column are monobasic; but the significance of this fact is lessened by the presence of the phthalates in the second column, and of the caprylates in the third.

The lower members of the acetic acid series permeate freely, but a sudden change occurs when the cœnanthylate and caprylate are reached. This would suggest that the increasing size of the molecule influenced the rate of absorption, but this does not hold good in other cases, for the malonate and succinate — the higher members of the oxalic acid series — are less cathartic than the lowest homologue, the oxalate.

The second column is even less homogenous than the third. The cœnanthylate may be looked upon as bridging the gap between the permeating simpler members and the purgative higher members of the acetic acid series, while the lactate and salicylate bear some relation to each other in both being oxy-acids. The phthalate and ethyl-sulphate, on the other hand, might have been expected in the third column, for the former is a dibasic salt, like most of the other salts of the third column, while the ethyl-sulphate might be expected to resemble the simple sulphate.

One curious relation, which struck us early in our experiments, and which determined to some extent the direction of our work, was that existing between the behavior of the ions in the intestine and the solubility of the corresponding calcium salts.

The solubility of some of these salts has not been determined, and we have therefore ascertained them by shaking the calcium salt in

water for three or four hours and estimating the amount of the salt dissolved in a given quantity of the filtered solution by evaporating and weighing. The results of the estimations made by others and by ourselves are given in the following table, in which the figures in the first column give the number of grams of salt dissolved in 100 c.c. water, while the figures in the second column give the temperature at which the estimation was made. We have selected temperatures at 40° C. where possible, so as to approach the conditions in the body more nearly.

TABLE II.

*Grams of Calcium Salt dissolved in 100 c.c. of water at the temperature given.*

	Grams	° C.		Grams	° C.
Calcium iodide <sup>8</sup> . . .	228.00	40.0	Calcium cœnanthylate <sup>7</sup> .	0.786	40.0
bromide <sup>8</sup> . . .	213.00	40.0	malate . . . .	0.753	21.5
chloride <sup>8</sup> . . .	104.00	35.0	ferrocyanide . .	0.580	23.0
nitrate <sup>8</sup> . . .	82.40	....	phthalate (ortho)	0.528	21.5
propionate <sup>3</sup> .	37.72	40.0	malonate <sup>2</sup> . .	0.422	40.0
acetate <sup>3</sup> . .	33.90	40.0	sulphate <sup>1</sup> . . .	0.210	38.0
formate <sup>3</sup> . .	17.40	39.7	caprylate . . .	0.133	21.5
butyrate <sup>6</sup> . .	16.30	40.0	citrate . . . .	0.089	21.5
lactate . . . .	....	....	tartrate . . . .	0.045	21.5
valerate <sup>4</sup> . .	8.20	40.0	hydric phosphate <sup>8</sup>	0.028	0.0
caproate <sup>5</sup> . .	2.50	40.0	fluoride <sup>8</sup> . . .	0.037	15.0
succinate <sup>2</sup> . .	1.15	41.6	oxalate . . . .	0.0	21.5
<sup>1</sup> RAUPENSTRAUCH: Monatshefte für Chemie, 1885, vi, p. 579. <sup>2</sup> MICZYNSKI: <i>ibid.</i> , 1886, vii, p. 255 <sup>3</sup> KRASNICKI: <i>ibid.</i> , 1887, viii, p. 595. <sup>4</sup> FÜRTH: <i>ibid.</i> , 1888, ix, p. 308. <sup>5</sup> KEPPICH: <i>ibid.</i> , 1888, ix, p. 589. <sup>6</sup> DEAZATHY: <i>ibid.</i> , 1893, xiv, p. 250. <sup>7</sup> LANDAU: <i>ibid.</i> , 1893, xiv, p. 707. <sup>8</sup> COMEY: Dictionary of chemical solubilities, London, 1896.					

On comparing Table I and Table II, it will be observed at once that the most soluble calcium salts are those formed by combinations with the indifferent ions (first column, table I), while the cathartic salts of the third column form very much less soluble salts with

calcium and the fluoride and oxalate (fourth column) are entirely insoluble. This is remarkably exemplified by the behavior of the acetic series, for while the first six members of this series are indifferent in the intestine and form fairly soluble salts with lime, the seventh (œnanthyllic) is slowly absorbed and rather insoluble, and the eighth (caprylic), which is very insoluble, acts in the same way as the sulphates. In the same way the least permeating member of the oxalic acid series (oxalic) forms an absolutely insoluble lime salt, while the less cathartic higher members form more soluble compounds with calcium.

Some exceptions to the general rule undoubtedly exist, apart from the nitrates, which we do not regard as of the same class as the others. Thus the succinate of calcium is more soluble than the œnanthylate, and yet sodium succinate is more cathartic, while the phthalates are less soluble<sup>1</sup> and yet appear in the second column. The lactate and salicylate also form rather soluble lime salts and yet appear to be somewhat slowly absorbed. Another exception is the ethyl-sulphate, which forms a very soluble lime salt, but it seems not impossible that this body may in part be decomposed in the course of absorption, in which case the sulphate formed would retard absorption. Similarly, the ferricyanide of calcium is soluble, but the sodium salt is reduced to the ferrocyanide in the intestine and therefore retards absorption.

It is to be remarked, however, that no very soluble lime salt is formed by the really cathartic group of ions (third and fourth columns, table I), while no acid forming insoluble lime salts is found in the first column. The exceptions cited above all fall into the second column, which is a makeshift group of substances neither entirely indifferent nor sufficiently slowly absorbed to entitle them to a place among the distinctly cathartic salts. Besides, it is very evident that the property which prevents the absorption of certain ions, and at the same time renders their combinations with lime insoluble, is not the only determining factor in absorption, for quinine hydrochlorate in traces prevents absorption. These exceptions therefore do not seem to us to invalidate the general result, namely, that acids which form insoluble salts with calcium act as

<sup>1</sup> The phthalates of calcium are said to differ considerably in solubility, but we found that the two phthalates precipitate lime water in the same degree of dilution. The quantity at our disposal did not admit of more accurate chemical examination.



cathartics when combined with ordinarily indifferent bases such as the alkalies.

The question at once arises whether the connection between these two properties is a causal one, *i. e.*, whether the cathartic salts are slowly absorbed because they precipitate calcium in the intestinal wall. It is needless to say that this is a possible explanation, for the precipitation of calcium has been shown to have a very considerable effect in such processes as the coagulation of the blood and of milk. The importance of calcium in the nutrition of the heart and of developing ova (Ringer), in the contraction of muscle (Locke), in the irritability of nerve fibres (Howell), and in the growth of plants (Loew) is generally recognized,<sup>1</sup> and we are tempted to suppose that in the absorption from the bowel the calcium plays a similar rôle. We feel however that our experiments are not sufficient to allow of a positive statement, and must leave the question open for the present. Howell's work on the action of oxalates on the heart left him in the same position of uncertainty as to whether the effects were due to a precipitation of calcium or to some specific action of the oxalates.<sup>1</sup> In this relation we may mention that in a number of experiments which we have performed on the tortoise heart the sulphate of sodium seemed to have the same effect as the acetate, while the citrate was extremely poisonous.

In the account of our results hitherto we have tacitly assumed that the salt failed to permeate the intestinal wall. This assumption is based upon results obtained by Hay, and more lately by Kovesi<sup>2</sup> and confirmed by our own observations that a considerable amount of the cathartic salt remains in the fluid in the intestine. Some salt undoubtedly disappears, but not nearly so much as when solutions of chloride of sodium or of any other indifferent salt are used. The depression of the freezing point ( $\Delta$ ) of the residue remains unchanged if the solution was originally isotonic, as were most of our solutions. If on the other hand a hyperisotonic solution is injected, the  $\Delta$  slowly declines to about .61 (that of the blood), while if a hypisotonic solution is used, a concentration of the fluid sets in until the  $\Delta$  again approaches that of the blood. This is in accord with Kovesi's results on the rabbit's intestine, but does not conflict as he supposes with Heidenhain's results obtained with sodium chloride

<sup>1</sup> HOWELL: *Journal of physiology*, 1894, xvi, p. 476.

<sup>2</sup> KOVESI: *Centralblatt für Physiologie*, 1897, xi, p. 553.

solutions, for the alteration in the  $\Delta$  of the intestinal contents is evidently due in both cases to the osmotic interchange of fluid and salt with the blood, which Heidenhain fully recognized.

In many of our experiments a considerable amount of mucus was present in the residual fluid, but this was not constant, and there did not seem more mucus in the residue of the cathartic solutions than in that of the standard solution. In many of the intestinal loops tape-worms were present, and in these there seemed more mucus than elsewhere. Hay<sup>1</sup> is inclined to look upon the increased secretion of mucus by the intestine under sodium sulphate as of some importance in retarding absorption, and this explanation has been again brought forward by Fusari and Marfori.<sup>2</sup> We are not disposed to look upon the secretion of mucus as of much importance in determining the absorption or non-absorption of the cathartic solutions.

The objection may always be brought against the method we have adopted that the conditions are so abnormal that no inferences as to the behavior of the uninjured intestine can be drawn. On the other hand no accurate results can be obtained by measuring the fluid in the fæces after the use of one of the purges, because the amount of fluid in the bowel previously is unknown. We have therefore attempted to determine the action of these purgatives by comparing the amount of fluid which escaped from a cæcal fistula after the administration by the stomach of isotonic solutions of various salts.

A medium sized dog was chloroformed, the abdomen laid open, and a loop of intestine immediately above the termination of the ileum sewed into the wound. Four days later, when complete adhesion had occurred, and the wound was rapidly healing, the loop was opened. A week later, the examination of the action of different salts was commenced. The animal received no food in the morning and in the afternoon a measured quantity of sodium chloride ( $\Delta .615$ ) was administered by the stomach tube and the amount of fluid passed by the fistula during the next hour measured. When no more fluid was passed an equal amount of an isotonic solution of another salt was given in the same way, and the fluid escaping by the fistula again measured. The results confirmed those obtained by the other method, but the investigation could not be carried far as the animal died, apparently from having been exposed to great cold during the night of Dec. 25.

<sup>1</sup> HAY: Saline cathartics, 1884, p. 69.

<sup>2</sup> FUSARI and MARFORI: Atti della acad. delle scienze med. e nat. in Ferrara, 1894; cited from Centralbl. f. innere Medicin, 1894, p. 1245.

Our results were as follows :

*Dec. 21, 1897.*

**Experiment 1.** Injected into stomach 100 c.c. NaCl  $\Delta$  .615.

15' Some solid matter evacuated with a few c.c. fluid.

60' Total amount discharged 5 c.c. fluid and faecal matter.

**Experiment 2.** Injected 100 c.c. sodium citrate  $\Delta$  .62.

15' Some faecal matter discharged and fluid began to appear.

60' Total amount of fluid discharged = 70 c.c.

*Dec. 22.*

**Experiment 1.** Injected 100 c.c. NaCl  $\Delta$  .615.

60' Total amount discharged = 4 c.c.

**Experiment 2.** Injected 100 c.c. sodium acetate  $\Delta$  .615.

60' Total amount discharged = 0.

**Experiment 3.** Injected 100 c.c. sodium phthalate (ortho)  $\Delta$  .62.

60' Total amount discharged = 0.

*Dec. 23.*

**Experiment 1.** Injected 100 c.c. NaCl  $\Delta$  .615.

60' Total amount discharged = 0.

**Experiment 2.** Injected 80 c.c.  $\left\{ \begin{array}{l} \text{sodium phthalate (ortho) } \Delta \text{ .62.} \\ \text{sodium phthalate (para) } \Delta \text{ .56.} \end{array} \right.$

60' Total amount discharged = 0.

*Dec. 24.*

**Experiment 1.** Injected 100 c.c. NaCl  $\Delta$  .615.

60' Total amount discharged = 0.

**Experiment 2.** Injected 100 c.c. Na<sub>2</sub>SO<sub>4</sub>  $\Delta$  .62.

60' Total amount discharged = 75 c.c.

The whole of the solutions of sodium chloride, sodium acetate, and sodium phthalate was absorbed in the course of its passage through the stomach and small intestine, while three fourths of the citrate and sulphate solutions reached the large intestine, and in the normal animal would have gone to increase the fluidity of its contents. We think that this demonstrates conclusively the method of action of the dilute solutions of the cathartics such as are found in some of the natural mineral waters. They do not necessarily increase the fluid of the bowel, but merely fail to be absorbed, and thus render the faeces more fluid and more easily moved through the large intestine.

## CONCLUSIONS.

1. The absorption of the salts of the fixed alkalies varies with the anion, those acids which form insoluble calcium salts tending to retard absorption more than others.

2. The behavior of these salts in the intestine has much in common with their action on unorganized colloid matter, as they tend to precipitate colloids in solution and are less imbibed than other salts by undissolved colloids.

3. But no complete analogy in their behavior towards the tissues in general exists, for several of the cathartic salts permeate the red corpuscles freely and others are absorbed rapidly from the serous membranes.

4. As regards the cations, ammonium is absorbed more rapidly than the fixed alkali ions, while those of the alkaline earths are very slowly taken up by the intestinal epithelium.

5. Dilute solutions (isotonic) of the saline cathartics retard the absorption of fluid from the stomach and small intestine, and thus act by rendering the contents more watery and more easily moved through the lower parts of the alimentary canal.

Protocols of the experiments are given on pages 429-434.

	Time in min- utes	Loop I.			Loop II.			Loop III.			Remarks
		Salt	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue	
<b>Experiment 1 — Nov. 1, 1897 — Cat.</b>											
a	30	NaCl	.59°	3.0 c.c.	MgSO <sub>4</sub>	.62°	15.5 c.c.	....	....	....	Length of each loop = 45 cm. Amount of solution injected into each loop = 15 c.c.
b	30	NaCl	.59	5.5	MgSO <sub>4</sub>	.62	15.0	....	....	....	
c	60	NaCl	.59	6.0	MgSO <sub>4</sub>	.62	25.0	....	....	....	
<b>Experiment 2 — Nov. 2, 1897 — Cat.</b>											
a	30	NaCl	.59	4.0	MgSO <sub>4</sub>	.62	9.0	....	....	....	Length of each loop = 45 cm. Amount of solution injected = 15 c.c.
b	30	NaCl	.59	8.0	Na <sub>2</sub> SO <sub>4</sub>	.62	14.0	....	....	....	
c	60	NaCl	.59	7.5	Na <sub>2</sub> SO <sub>4</sub>	.62	14.0	....	....	....	
<b>Experiment 3 — Nov. 4, 1897 — Cat.</b>											
a	30	NaCl	.59	3.0	NaOxalate	.625	9.5	....	....	....	Length of each loop = 45 cm. Amount of solution injected = 15 c.c.
b	40	NaOxalate	.625	17.0	NaCl	.59	13.0	....	....	....	
c	30	Na Tartrate	.625	27.0	NaCl	.59	19.5	....	....	....	
<b>Experiment 4 — Nov. 8, 1897 — Cat.</b>											
a	30	NaAcetate	.62	8.5	NaCl	.59	10.0	....	....	....	Length of each loop = 45 cm. Amount of solution injected = 15 c.c.
b	30	NaCl	.59	11.5	NaAcetate	.62	10.0	....	....	....	
<b>Experiment 5 — Nov. 9, 1897 — Cat.</b>											
a	30	NaCl	.59	0.0	NaTartrate	.625	9.0	....	....	....	Length of each loop = 45 cm. Amount of solution injected = 15 c.c.
b	30	Na Tartrate	.625	10.0	NaCl	.59	10.5	....	....	....	



Time in min- utes	Loop I.			Loop II.			Loop III.			Remarks
	Salt	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue	
Experiment 10 — Nov. 19, 1897 — Cat.										
a	30 NaCl	.615°	2.0 c.c.	NaCl	.615°	4.0 c.c.	....	....	....	Length of each loop = 45 cm. Amount injected = 15 cc. Loops washed with normal salt solution
b	30 NaH <sub>2</sub> PO <sub>4</sub>	.56	7.0	Na <sub>2</sub> HPO <sub>4</sub>	.56	8.0	....	....	....	
c	30 NaCl	.615	8.0	Na <sub>2</sub> HPO <sub>4</sub>	.56	15.5	....	....	....	
Experiment 11 — Nov. 29, 1897 — Dog.										
a	30 NaAcetate	.615	1.5	NaFormate	.63	4.0	NaPropionate	.66°	7.0 c.c.	Length of each loop = 30 cm. Amount injected = 25 c.c.
b	30 NaAcetate	.62	1.5	NaButyrate	.52	2.0	NaValerate	.69	7.0	
c	30 NaCaprylate	.61	24.0	NaCaproate	.62	4.0	NaAcetate	.615	10.0	
d	35 NaLactate	.62	15.0	NaLactate	.62	7.0	NaAcetate	.615	3.0	
Experiment 12 — Dec. 1, 1897 — Dog.										
a	30 NaCl	.615	6.0	NaCl	.615	2.75	NaCl	.615	1.0	Length of each loop = 30 cm. Amount injected = 25 c.c. Loops washed with normal salt solution.
b	30 NaCl	.615	13.0	NaPhthalate (ortho)	.62	10.5	NaPhthalate (para)	.56	7.0	
c	30 NaCl	.615	9.0	NaCl	.615	5.0	NaCl	.615	2.5	
d	30 NaCl	.615	8.0	NaPhthalate (ortho)	.62	12.0	NaPhthalate (para)	.56	11.0	
Experiment 13 — Dec. 3, 1897 — Dog.										
a	30 NaCl	.615	15.0	NaCl	.615	13.0	NaCl	.615	16.0	Length of each loop = 30 cm. Amount injected = 25 c.c.
b	30 NaCl	.615	17.0	NaPropionate	.66	14.5	NaValerate	.625	11.5	
c	30 NaCl	.615	16.0	NaButyrate	.52	7.0	NaTartrate	.625	21.0	





Loop I.				Loop II.				Loop III.				Loop IV.			
Time in min- utes	Salt	$\Delta$	Residue	Salt	$\Delta$	Residue	Salt	$\Delta$	Residue	Salt	$\Delta$	Residue	Salt	$\Delta$	Residue
<b>Experiment 18</b> — Feb. 10, 1898 — Dog.															
<i>a</i>	30	NaCl	64°	7.25 c.c.	NaCl	64°	3.25 c.c.	NaCl	64°	5.0 c.c.	NaCl	61°	2.0 c.c.		
<i>b</i>	30	NaCl	64	9.0	NaNO <sub>3</sub>	65	15.0	KNO <sub>3</sub>	63	14.5	NaI	62	9.0		
REMARKS. — Length of each loop = 30 cm. Amount injected = 25 c.c.															
<b>Experiment 19</b> — Feb. 15, 1898 — Dog.															
<i>a</i>	30	NaCl	64	3.0	NaCl	64	2.5	NaCl	64	5.0	NaCl	64	2.5		
<i>b</i>	30	NaCl	64	3.0	NaBr	60	5.5	NaI	60	20.0	NaI	62	15.0		
<i>c</i>	30	NaI	62	8.0	NaCl	64	2.5	NaCl	64	26.0	NaCl	64	2.5		
<i>d</i>	30	NaCl	64	5.0	NaSalicylate	62	9.0	NaCl	64	9.0	NaCl	64	2.5		
REMARKS. — Length of each loop = 30 cm. Amount injected = 25 c.c.															
<b>Experiment 20</b> — March 1, 1898 — Dog.															
<i>a</i>	30	NaCl	64	8.0	NaCl	64	8.5	NaCl	64	9.5	NaCl	64	9.5		
<i>b</i>	30	NaCl	64	8.0	K(C <sub>2</sub> H <sub>5</sub> )SO <sub>4</sub>	63	18.0	Na <sub>2</sub> SO <sub>4</sub>	60	27.5	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	58	21.0		
<i>c</i>	30	NaCl	64	8.0	NaCl	64	19.0	NaCl	64	6.0	NaCl	64	6.0		
<i>d</i>	30	NaCl	64	5.5	....	....	....	NaCl + Quin- ine HCl	....	20.0	NaCl + Quin- ine HCl	....	19.0		
REMARKS. — Length of each loop = 30 cm. Amount injected = 25 c.c. KC <sub>2</sub> H <sub>5</sub> SO <sub>4</sub> contains free SO <sub>4</sub> .															
<b>Experiment 21</b> — March 15, 1898 — Dog.															
<i>a</i>	30	NaCl	625	3.0	NaCl	625	1.0	NaCl	625	1.0	NaCl	625	2.0		
<i>b</i>	30	NaCl	625	4.5	Na(C <sub>2</sub> H <sub>5</sub> )SO <sub>4</sub>	62	8.0	Na <sub>2</sub> SO <sub>4</sub>	835	50.0	Na <sub>2</sub> SO <sub>4</sub>	835	45.0		
<i>c</i>	30	NaCl	625	4.0	NaCl	625	1.5	NaCl	625	7.0	NaCl	625	5.5		
<i>d</i>	30	KFerrocyanide	63	27.0	KFerrocyan.	63	22.0	NaCl	625	5.5	NaC <sub>2</sub> H <sub>5</sub> SO <sub>4</sub>	62	23.0		
REMARKS. — Length of each loop = 40 cm. Amount injected = 30 c.c.															



## THE MOVEMENTS OF THE FOOD IN THE ŒSOPHAGUS.

By W. B. CANNON AND A. MOSER.

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THE movements of deglutition, in common with many other physiological processes, were explained by the older physiologists on anatomical grounds. Thus Magendie<sup>1</sup> divided the act into three parts, corresponding to the anatomical regions of the mouth, pharynx, and œsophagus. The muscles of each of these divisions were considered the active agents in propelling the food onward. The function of moving the mass to the pharynx was variously ascribed to the tongue itself, to the mylohyoid muscles, and to gravity. For the second part, the movement through the pharynx, there was more unanimity of opinion, since the constrictors, especially the middle and lower, were evidently concerned.

Direct observations on the movement of swallowed masses in the œsophagus were first made by Mosso.<sup>2</sup> The œsophagus of a dog was laid bare and a transverse incision made through it, or a piece of it excised. A small wooden ball was placed in the canal below the excised part, and the animal was then stimulated to swallow. One or two seconds after the contraction of the pharyngeal muscles a peristaltic wave began to traverse the œsophagus. This wave did not stop at the point of excision, but in due time reappeared below and carried the ball to the stomach. Thus the act was shown to be controlled by the central nervous system. Peristalsis was so plainly the motive power that the action was never doubted. Yet this belief was soon to be questioned.

In 1880, Falk and Kronecker<sup>3</sup> studied the movements in the mouth and pharynx, and advanced the theory that deglutition was accomplished by the rapid contraction of the muscles of the mouth. During the act of swallowing the air-tight buccal cavity shows a manometric pressure of 20 centimetres of water. The same pressure was demonstrated to be present also in the œsophagus, but not in

<sup>1</sup> MAGENDIE: *Précis élémentaire de physiologie*. Paris, 1836, i, p. 63.

<sup>2</sup> MOSSO: *Moleschott's Untersuchungen*, 1876, xi, p. 331.

<sup>3</sup> FALK AND KRONECKER: *Archiv für Physiologie*, 1880, p. 296.

the stomach. This pressure was considered sufficient to force food through the œsophagus before the peristaltic wave traversed it. Another argument for rapid descent was found in the fact that cold water can be felt in the epigastric region almost immediately after being swallowed. Further, when strong acids pass through the gullet, they corrode but small parts of it, and not the entire mucous membrane, as would be the case were the acid carried to the stomach by peristalsis.

In the same year, in confirmation of the above results the well known experiments of Kronecker and Meltzer<sup>1</sup> were published. A rubber balloon, connected by a tube to a Marey tambour, was placed in the pharynx, and another balloon, similarly connected, was introduced a varying distance into the œsophagus. When water was swallowed the increased pressure in the pharynx was transmitted to the first tambour, which traced a curve on a rotating drum. Almost instantly thereafter the œsophageal balloon was compressed, causing the second tambour to write its curve below the first. This second curve was supposed to mark the passage of the food through the œsophagus. After a varying number of seconds the œsophageal balloon recorded another curve, caused by a peristaltic wave which carried to the stomach any fragments left in the canal.

To demonstrate that the first curve of the œsophageal balloon was caused by the passage of the swallowed liquid, Meltzer devised another experiment. A strip of blue litmus paper was placed in a stomach tube, opposite the side openings at the lower end. A long thread attached to the paper ran through the tube to the other end. The tube was now passed into the lower end of the œsophagus and an acid drink swallowed. If the litmus paper was pulled away from the side openings a second after the beginning of swallowing, it was found distinctly reddened, showing a rapid descent of the swallowed liquid. Reference to this experiment will be made later.

From these observations Kronecker and Meltzer concluded that liquids and semi-solids are not carried to the stomach by peristalsis, but are squirted down the œsophagus by the rapid contraction of the muscles of the mouth. For this purpose the mylohyoids alone are sufficient, since the middle and inferior constrictors can be cut without interfering with the act. The succeeding peristalsis is of use merely in gathering up adhering fragments and carrying them to the stomach.

<sup>1</sup> KRONECKER AND MELTZER: *Archiv für Physiologie*, 1880, p. 446.

To determine whether the cardia offered any resistance to this rapid passage into the stomach, Meltzer<sup>1</sup> tried another method. If a stethoscope is placed over the epigastrium during the swallowing of liquids, a sound can be heard from six to seven seconds after the rise of the larynx. The sound is caused by the passage of the swallowed mass, liquid and air, through the tonically contracted cardia. In a few cases the sound is heard immediately after swallowing, showing a probable insufficiency of the cardia. These phenomena led Kronecker and Meltzer<sup>2</sup> to modify their previous views. They now maintained that the mass is not squirted by the mylohyoids directly into the stomach, but halts a short distance above the cardia. Here it remains until carried into the stomach by the succeeding peristalsis, about six or seven seconds after the beginning of swallowing.

The care with which these experiments were conducted has won general assent to their results. But the methods employed are not beyond criticism. Primarily it may be said that swallowing with one or more balloons and a stomach tube in the canal is not normal deglutition. Moreover, semi-solids were found to yield less readily to pressure than liquids, and even to be delayed in their descent.<sup>3</sup> Again nearly all the work was done with liquids and semi-solids; solids are not even mentioned. The investigators themselves declared that their results were true for liquids and semi-solids only, and admitted that a dry bolus could not be so swallowed. Yet the indiscriminate use of such terms as "liquid," "swallowed mass," and "bolus," easily leads to an inference that the results of these investigations are true for the swallowing of food of all consistencies. A difference in rate, however, certainly exists in respect to consistency, and it was to discover the actual movement of solids, semi-solids, and liquids in the normal œsophagus that the present work was undertaken.

Over a year and a half ago it was suggested by Prof. H. P. Bowditch that if some substance opaque to the Röntgen rays were swallowed, it could be seen in its passage to the stomach and the nature of its movement thus determined. Anæsthesia could be dispensed with, — a desirable condition, since observers had found that it inter-

<sup>1</sup> MELTZER: Centralbl. für die med. Wissenschaften, 1883, p. 1.

<sup>2</sup> KRONECKER AND MELTZER: Archiv für Physiologie, 1883, Suppl. Bd., p. 337, 351.

<sup>3</sup> KRONECKER AND MELTZER: *ibid.*, p. 337.

ferred greatly with the deglutition reflex.<sup>1</sup> It would be unnecessary to open either the abdominal or the pleural cavity. The reflex stimulus of food moreover would be better than electrical stimulation of the superior laryngeal nerve. In short, the animal would swallow normal food under practically normal conditions. At Dr. Bowditch's suggestion and with his valuable assistance — which we gratefully acknowledge — we made the following series of experiments.

To render the swallowed mass opaque subnitrate of bismuth was used. The salt is tasteless, practically inert, and can be fed in large quantities without harm. In order that observations could be made by more than one person, all experiments were conducted in a dark room. On the side of the animal opposite the Crookes tube was placed an open fluorescent screen on which the different tissues of the animal were outlined with varying degrees of light and shade. Among these shadows the swallowed mass appeared as a darker object, and thus its motion could be studied.

For the first experiments the goose was selected. The head and neck were held stationary by a tall pasteboard collar which allowed free movement of the head without constriction of the neck. The fluorescent screen was placed against this collar at a uniform distance of thirty centimetres from the tube. When a bolus of corn meal mush mixed with bismuth was placed in the pharynx it descended slowly and regularly, and occupied about twelve seconds in passing over a distance of fifteen centimetres. The screen was marked at intervals of two centimetres with cross lines, by means of which the relative rate in different parts of the œsophagus could be studied. A vibrator marking tenths of a second was interrupted whenever the bolus crossed a line. An average of over one hundred such observations showed that the rate became slightly slower as the bolus proceeded.

In order to test liquids, molasses was mixed with bismuth to such a consistency as to drop easily from a glass rod. When this was fed with a pipette it passed slowly and regularly down the œsophagus, clearly by peristalsis. The rate was about the same as for solid food. In both these experiments, the addition of water would sometimes cause irregularities in the descent. Microscopic sections from four different parts of the œsophagus of the goose showed no histological difference.

In the experiments on the cat, the animal was placed on its back and

<sup>1</sup> MELTZER: *Journal of experimental medicine*, 1897, ii, p. 457.

left side on a holder. The extremities were secured by straps. The head was held between two upright rods connected above by a thong; this allowed free movement of the head without resistance to the passage of food. Shreds of meat dipped in bismuth were ordinarily masticated and swallowed without difficulty. For soft solids bread and milk were used, so fluid as to be easily drawn up into a pipette. The insolubility of the bismuth salt rendered the study of liquids more difficult. Strong solutions of potassic iodide and other salts and suspension of bismuth in acacia and molasses were tried; but a simple mixture of milk and bismuth, shaken in a test tube and immediately drawn up into a pipette, was found most practicable.

Inasmuch as the movement of these different foods varied in different parts of the œsophagus, it will be convenient to divide the latter into three sections. The first or cervical portion extends from the pharynx to the thorax, the second or thoracic from here to the lower half of the heart, and the third comprises the rest of the canal. The relative length of these three parts is about in the ratio of 9:8:6.

The beginning of deglutition was noted by one observer by a finger on the larynx; the same observer called out when the bolus arrived at the thorax, heart, and stomach respectively, while the other observer noted the time. The movement of solids will first be considered. The descent the entire way was by peristalsis, but the rapidity varied. The duration of the movement in the cervical portion was two and a half seconds, and in the thoracic region a little less than two seconds. At the lower end of the heart there was sometimes a slight pause. In the lower section, from the heart to the stomach, the movement was decidedly different. The rate was always very slow. The distance was less than one-third of the entire canal, yet the time consumed in this part ranged from six to seven seconds, or three-fifths of the entire time of descent. The character of the movement here was also peculiar. Whereas in the upper sections the passage was uniform and regular, with a slight acceleration in the thoracic region, here it was apparently irregular, for the bolus descended about one centimetre with each inspiratory movement of the diaphragm, and remained stationary or descended very slightly during expiration. Thus a series of hitches seemed to carry the bolus to the cardia. A probable explanation of this peculiar movement is that the stomach and lower œsophagus were pulled down with each descent of the diaphragm. This would make the movement appear irregular although it was really a slow peristalsis.

It may be well to remark here that this movement was invariably observed in the cat with every kind of food.

Semi-solids, namely, a mush of bread and milk, descended in the same way as solids; but the rate was slightly faster in the upper œsophagus, for the bolus took about a second less to reach the cardiac level. From here the rate was the same as with solids.

For liquids one and a half to two seconds sufficed for the descent to the midheart region. Here there often occurred a long pause — from a few seconds to a minute or more. Then the œsophagus apparently contracted above the liquid, which slowly passed on to the stomach as already described. Sometimes it seemed as if a swallowing movement, evidenced by a rise of the larynx, started the peristaltic wave. Again, several swallows would succeed one another before the liquid passed on. A few times the bismuth and milk seemed strung out along the œsophagus; some more liquid descending would gather this up, and the whole mass assuming an ovoid form would move into the stomach.

Thus in the cat the total time for deglutition varies from nine to twelve seconds. The lowest section presents no change ascribable to a difference in consistency, while in the upper sections the rate does slightly increase with the more liquid character of the food.

In experiments on the dog, bismuth enclosed in capsules or wrapped in shreds of meat was fed as the solid. The general phenomena were as follows. With the rise of the larynx there was a quick propulsive movement of the bolus, which descended rapidly for a few centimetres, sometimes as far as the clavicle. From this point the rapidity was diminished; yet no pause was observed; the bolus simply moved more slowly. This rate was then continued to the stomach without a slackening of speed in the diaphragmatic region, as was observed in the cat. Semi-solids moved in the same way as solids. The total time of descent from larynx to stomach was from four to five seconds.

Liquids gave even a more decided squirt in the beginning of the movement. To render the œsophagus as lax and free as possible, the head of the dog was released from the upright rods and held by the hands after the food was placed in the mouth. Sometimes the liquid descended rather rapidly as far as the heart, at other times no further than the clavicle; then without a pause it passed on slowly and regularly, reaching the stomach in about the same time as solids and semi-solids.



Thus in the dog and cat but little variation was seen in the swallowing of liquids and solids. The liquids pass somewhat faster in the upper œsophagus. But in some animals the difference of rate with foods of varying consistency is much more marked. In the horse, for instance, mere observation shows a decided variation in the rate of movement in the œsophagus. Liquids shoot along the gullet, while solids move clearly by peristalsis. To determine the rate of solids one hand was placed on the larynx of a horse to note the beginning of swallowing and the other hand near the shoulders, where the bolus could be easily felt in its passage. The time consumed by the bolus in passing over a certain distance was measured by a stop watch. The rate obtained for solids, such as hay or grain, was from thirty-five to forty centimetres a second.

For semi-solids, a mixture of bran and water was made, thin enough to run easily between the fingers. Each bolus was watched by a separate observer with a separate watch. The average rate obtained was the same as for solids.

Liquids in the horse pass with a rapidity too great to be affected by peristalsis. Another force must be sought. Among the various muscles supposed to be effectual in moving food into the pharynx, the mylohyoids were shown by Meltzer<sup>1</sup> to be essential. The styloglossi were cut by him without much interference with deglutition, but section of the mylohyoid nerves rendered the act impossible. The activity of these muscles in the horse during swallowing is easily perceived by the hand. Their energetic contraction is a sufficient explanation of the rapid passage of water through the œsophagus. The motion here is more than five times as rapid as that of solids and semi-solids.

Meltzer's experiment to measure the rate of liquids in man by passing a stomach tube containing litmus paper was repeated by us with some modifications. Congo red paper was used, since it is more sensitive than litmus; it also furnishes a means of differentiating between mineral and organic acids, as the discoloration produced on Congo red by mineral acids is removed by ether. It was thus possible to distinguish between the discoloration produced by gastric regurgitation and that produced by the swallowed liquid. For the swallowed liquid one-half per cent lactic acid was found most satisfactory, as the color produced by it on Congo red test paper is almost instantly discharged in ether. By this method the paper

<sup>1</sup> KRONECKER and MELTZER: *Archiv für Physiologie*, 1880, p. 299.

was found discolored within half a second after the rise of the larynx, certainly too short a period for a peristaltic wave to carry the liquid to the neighborhood of the cardia.

The X-ray method lends itself less successfully to the study of deglutition in man than in the other animals we have studied. The thickness of the thorax, the distance of the œsophagus from the surface, and the relation to dense tissues, render the observation of a swallowed mass difficult, especially when the mass is in rather rapid motion. The few observations which we have to report were made on a seven year old girl placed in the sitting posture. Gelatine capsules containing bismuth were used for solids, and were traced to a point below the heart. The motion was very regular, and apparently due to peristalsis, for the bolus descended without a hitch or irregularity of any kind. Sometimes the capsule became fixed in the upper œsophagus at about the level of the second rib. Repeated swallows of water would fail to dislodge it. An interesting point was noted here. With each attempt at swallowing, the capsule would rise slightly as if the œsophagus was pulled up with the rise of the larynx; then the capsule would descend to its former position.

Semi-solids—a mush of bread and milk—could be seen about as far as solids, *i. e.* to just below the heart. The motion of the mushy bolus was the same as with solids, except that the rapidity was perhaps slightly greater.

It should be noted here that with the human subject, as well as with the horse, our results for semi-solids differ from those derived by Meltzer's method; for according to his statements semi-solids, like liquids, are squirted down the œsophagus and are not propelled by peristalsis, as has been the case in our observations.

Liquids—bismuth and water—were seen only in the neck and upper thorax. Here there was a decided squirt. With the rise of the larynx the liquid was seen to pass rapidly through the pharynx and well down into the thoracic œsophagus before it was lost to observation. The rate, however, by estimation was less than that of liquids in the horse.

There remains to be considered Meltzer's latest investigation,<sup>1</sup> in which he endeavored to ascertain whether liquids remain above the cardia till the arrival of the peristalsis, or ooze down before. An experimental answer was secured by Meltzer by the following

<sup>1</sup> MELTZER: *Journal of experimental medicine*, 1897, ii, p. 453.

method. The abdominal and gastric walls of an anæsthetized dog were incised and a tube (vaginal speculum) introduced. Through this the entrance of food into the stomach could be observed directly. In repeated experiments no liquid was seen to pass through the cardia before the arrival of the peristaltic wave. An incision through the diaphragm near its anterior origin showed that the swallowed liquid was not squirted as far as a point an inch above the diaphragm. To observe the œsophagus nearer its beginning, the upper three ribs were resected on the left side. Thus the swallowed liquid was seen to shoot along the œsophagus before any peristalsis reached this point. The resection of the fifth rib exposed the œsophagus half way between the bifurcation of the trachea and the diaphragm. Here a bulging was sometimes observed immediately after the beginning of the act, and the swallowed mass remained there until a peristaltic wave carried it down. If the mass swallowed was small, or was projected with moderate force, it might not even reach as far as the bifurcation. From these experiments Meltzer concluded that in animals as in man, liquid food is not carried down the œsophagus by peristalsis, but is thrown rapidly into a deep part of the canal. The depth reached depends on the quantity swallowed, the force used, and the tonicity of the lower part of the œsophagus.

The difference between these methods of Meltzer and those employed in our experiments has already been mentioned; and merely his results, which were obtained with liquids alone, need be considered here. According to our observations on the dog, there was no distinct pause at any part of the canal. The movement simply became slower, and continued at this rate until the stomach was reached. Neither was the rate through the diaphragmatic part of the œsophagus slower than through the thoracic. The quick propulsive movement noticed in the dog was observed with solids and semi-solids as well as with liquids, but the liquids descended further down the canal before the movement changed to the slower peristalsis. While this difference was evident to the eye, the total time consumed by liquids in passing from pharynx to stomach was not enough shorter than the time for solids and semi-solids to be determined by our measurements.

## SUMMARY.

The phenomena of œsophageal deglutition as determined by our experiments may then be described as follows: —

There is a difference in swallowing according to the animal and the food which is used.

In fowls the rate is slow and the movement always peristaltic, without regard to consistency. A squirt-movement with liquids is manifestly impossible, as the parts forming the mouth are too hard and rigid. With this diminution of propulsive power in the mouth there is observed a greater reliance on the force of gravity. The head is raised each time after the mouth is filled, and the fluid by its own weight trickles into the œsophagus, through which it is carried by peristalsis.

In the cat the movement is always peristaltic and slightly faster than in fowls. A bolus takes from nine to twelve seconds in reaching the stomach. Liquids move somewhat more rapidly than semi-solids in the upper œsophagus. In the lower or diaphragmatic part the rate is very much slower than above, and is the same for liquids as for solids.

In the dog the total time for the descent of a bolus is from four to five seconds. The food is always propelled rapidly in the upper œsophagus and moves more slowly below. This rapid movement is frequently continued further with liquid food. No distinct pause was observed when the movement of the bolus changed from the rapid to the slower rate.

In man and the horse liquids are propelled deep into the œsophagus at a rate of several feet a second by the rapid contraction of the mylohyoid muscles. Solids and semi-solids are slowly carried through the entire œsophagus by peristalsis alone.

# A CONTRIBUTION TO THE CHEMISTRY OF CYTOLOGICAL STAINING.

By ALBERT MATHEWS.

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IT has long been known to histologists that different elements of the cells and tissues show affinity for different stains. Many nuclei, some mucins, and hyaline cartilage stain powerfully in methyl green, Bismarck brown, thionin, and other basic stains, while other nuclei and most cytoplasmic elements show a decided preference for eosin, acid fuchsin, acid green, and other acid stains. The nature of the chemical reactions upon which this elective staining power rests has never received adequate attention. It is several years since Ehrlich<sup>1</sup> classified all stains as "acid," "basic," and "neutral," yet it is still uncertain upon just what properties the affinity of chromatin for basic dyes and cytoplasm for acid dyes really depends. It is still not uncommon to find in cytological works methyl green and other basic stains regarded as microscopical reagents for the detection of chromatin, and some cytoplasmic bodies because of their affinity for basic dyes have been looked upon as chromatin or derivatives of chromatin.

The first observations on the possible chemical basis of the staining reactions of chromatin were made by Miescher,<sup>2</sup> who found that nucleinic acid, a component of chromatin, formed green insoluble salts with methyl green. Lilienfeld<sup>3</sup> called attention to the same fact and referred the affinity of the chromatin for basic stains to the formation of these salts. Lilienfeld<sup>4</sup> also advanced our knowledge by showing that albumin stained pre-eminently in the acid stains and nucleinic acid only in the basic. In studying the artificial nucleins he found that they possessed a varying affinity for acid or basic stains according as the nucleinic acid was more or less completely saturated with albumin. He also observed that egg albumin precipitated by

<sup>1</sup> EHRLICH: *Archiv für Physiologie*, 1879, p. 571.

<sup>2</sup> MIESCHER: *Verhandl. d. naturf. Gesellsch. in Basel*, 1874, vi, p. 138.

<sup>3</sup> LILIENFELD: *Archiv für Physiologie*, 1893, p. 391.

<sup>4</sup> LILIENFELD: *Archiv für Physiologie*, 1893, p. 554.

alcohol stained neither in acid nor in basic stains. Lilienfeld believed that the affinity of the cytoplasm for acid stains was due to its containing much albumin, but he made no suggestion as to the nature of the combination of the stain with the albumin molecules. He fell into error in supposing that albumin stained only in the acid stains. It will be shown farther on that under suitable conditions the albumin molecule may be made to combine also with the basic stains. In practical experience histologists have observed that sections stained in acidified solutions of the Biondi-Ehrlich mixture take chiefly the acid stain, while in alkaline solutions they take the basic. No explanation of the cause of this phenomenon has been offered, so far as I am aware.

The present paper presents the results of experiments which give some indication I believe of the probable nature of these affinities, and which also show how far cytological stains may be used as accurate micro-chemical reagents. It must be understood at the outset that the results here recorded of experiments on egg albumin, albumoses, and peptones can be directly applied only to such sections of tissues as have been killed and fixed in alcohol or acid media free from metallic salts such as mercuric and platinic chlorides; and further that the conclusions do not apply to those staining processes which probably involve the precipitation of the coloring matter in the tissue; such as the iron-hæmatoxylin method.

## I. EXPERIMENTS ON ALBUMOSES.

A. **The acid stains.** — Physiological chemists are aware that albumin and the albumoses react like weak bases, and that they will combine with free acids. If acetic, hydrochloric, or sulphuric acid is added to a solution of albumoses it may be shown by appropriate methods that the acids have chemically combined with the albumoses, although no precipitate is thrown down. Many other free acids enter into similar combinations with the albumins and albumoses, but form insoluble compounds, thus precipitating the albumoses from solution. If a solution of picric acid is brought into a solution of albumose a precipitate consisting of the picric acid combination of the albumose is thrown down. The same kind of reaction ensues with meta-phosphoric, molybdic, wolframic, phosphor-wolframic, tannic, stearic, or chromic acid. Only the free acids will combine with the albumoses. If a neutral solution of the salts of the above-mentioned acids is added to a neutral solution of the albumoses no

reaction occurs. A few drops of acetic or hydrochloric acid are necessary to call forth the reaction. On the addition of acetic acid to a mixture of albumose and sodium picrate, a precipitate consisting of the picric acid combination of albumose appears at once. Probably the reason is that the acetic acid sets free the picric acid, which at once combines with the albumose molecule.

It occurred to me that the so-called "acid" stains, which are generally the sodium salts of sulfonic acids, probably combine with the albumin molecule in the same manner as the above mentioned acids. Experiment fully bore out this hypothesis, for I found that the acid stains possess the same albumin-precipitating powers as sodium picrate or wolframate. The addition of acid fuchsin, acid green, nigrosin, anilin blue black, erythrosin, congo red, carminate of soda, methyl blue, indigo carmine, or other acid stain to a solution of albumoses or albumin gives no reaction. If, however, a few drops of dilute acetic acid be added to the mixture of albumose and acid stain, the colored combination of the stain with the albumose is at once precipitated. One can indeed use this test for detecting the presence of albumin or albumoses in solution or for distinguishing between acid and basic stains, as the basic stains do not give this reaction. This reaction of the acid stains indicates beyond doubt that these stains when in acidulated solutions will enter into chemical combination with the albumose or albumin molecule like any other acid. Inasmuch as it is probable that the free acids enter one or more of the basic  $\text{NH}_2$  groups of the albumin molecule, the acid stains also probably enter this group.

**B. The basic stains.** — The basic stains react with the albumoses very differently from the acid. The former stains are generally the chlorides or hydrochlorates of colored organic bases. They react as might be expected like other organic bases. It is well known that in alkaline solution many metals may be made to form combinations with the albumin molecule. If for instance lead acetate is brought into a neutral solution of albumoses or albumins no reaction occurs. If now the solution be made slightly alkaline with sodium carbonate a precipitate is formed consisting of a lead compound of albumin. It is probable that the lead enters the albumin molecule in a different place from the acids already mentioned, and that it enters the hydroxyl of the phenol group, since gelatine and protamin, which lack this group, are not precipitated by basic lead acetate. Many organic bases react like lead.

Protamin, histon, and quinine — strong organic bases — precipitate albumin and the albumoses in alkaline solution. The basic aniline colors react similarly. They may in this manner be made to form colored combinations with albumin and the albumoses.

If basic fuchsin, methyl green, thionin, safranin, or other basic stains (with the possible exception of vesuvin), are brought into a neutral or slightly acid solution of the albumoses, no reaction takes place. If on the other hand they be brought into solutions of the albumoses made slightly alkaline with sodium carbonate a flocculent, colored precipitate consisting of the albumose in combination with the dye is thrown down. This reaction may be used to distinguish the basic from the acid dyes. Vesuvin is precipitated by sodium carbonate alone, but if albumoses are present it is possible, though I have not specially examined the matter, that the precipitate is a combination of vesuvin with the albumose.

These experiments prove that many of the basic dyes enter into chemical combination with the albumose molecule when in alkaline solutions, forming insoluble colored compounds. They show also that in acid or neutral solution this reaction does not occur.

To sum up: (1) The acid stains will combine with albumoses only in acid solutions. (2) Under such circumstances they form combinations similar to picric or other acid combinations with albumoses, and probably enter one or more  $\text{NH}_2$  groups in the albumose molecule. (3) The basic stains will combine with the albumoses only in alkaline solution, when they form insoluble colored compounds. The basic dyes react in this respect like basic lead acetate, protamin, histon, or other organic bases. (4) The basic stains probably enter the hydroxyl of the phenol group of the albumose molecule, since they will not precipitate gelatine.

## II. COAGULATED EGG ALBUMIN.

A. **The acid stains.**—Coagulated egg albumin reacts toward the acid stains like the albumoses. If egg albumin coagulated by heat or alcohol be brought into neutral or alkaline solutions of the acid dyes the albumin will not stain. It is true that it will imbibe a certain amount of color and will appear stained, but this color is easily and quickly removed by washing in water. If on the other hand pieces of coagulated albumen be brought into solutions of the acid stains which have been slightly acidulated with acetic acid the albumin



stains instantly and intensely. The color cannot be removed even by prolonged washing. A most striking contrast is shown by two pieces of coagulated albumin, one of which has been immersed in a neutral, the other in an acid solution of acid fuchsin. After washing, the former will be found to be colorless, the latter a brilliant red.

**B. The basic stains.**—Towards the basic stains coagulated albumin reacts on the whole like the albumoses. Egg albumin coagulated by heat is normally alkaline. If its alkalinity be neutralized or if it be brought into a slightly acid solution of the basic dyes it will stain but slightly. Its power of staining under such circumstances I believe to be due to some other constituent than the albumin, possibly to the mucoid matter present. If however the coagulated egg albumin without neutralization be brought into neutral or slightly alkaline solutions of the basic dyes methyl green, thionin, safranin, methylen blue, or toluidin blue, it stains with great intensity and instantaneously. This may be most strikingly seen in the case of thionin or safranin. If two pieces of coagulated egg albumin be brought the one into slightly acid and the other into alkaline solutions of thionin, the stain poured off after a few seconds, and the albumin washed in water, the piece that has been in the alkaline solution will be an intense purple, the other barely tinged with color.

These reactions clearly indicate that the staining of coagulated albumin depends on chemical combinations similar in all respects to those which the albumoses enter into with the same stains. In neutral solution, neutral coagulated albumin combines neither with acid nor basic stains; in alkaline solutions, it combines only with the basic; in acid solutions, only with the acid stains.

### III. CARMINIC ACID, HÆMATINE, AND THE ACTION OF ALUMINIUM.

Paul Mayer<sup>1</sup> has shown that carminate of soda and hæmatine are plasma stains, as are the acid aniline colors, whereas the aluminium salts of these acids are chromatin stains, as are the basic aniline colors. Carminate of soda and hæmatine react towards the albumoses like the acid stains. In neutral or alkaline solutions they do not combine with the albumoses or albumins; in acid solution they precipitate the albumoses at once. Freshly prepared hæmatoxylin will not stain tissues, and corresponding with this I find that fresh solutions

<sup>1</sup> MAYER: Mittheil. a. d. zoolog. Stat. Neapel, 1892, x, p. 170.

of hæmatoxylin will precipitate the albumoses neither in acid, neutral, nor alkaline solutions. As aluminium gives carminic acid and hæmatine the staining properties of the basic aniline colors, it is of interest to see how these salts react towards the albumoses. I found that the aluminium salts of these acids (Mayer's carmalaun and hæmalaun) would not precipitate the albumoses in neutral or acid solutions. Thus they differ completely from the sodium salts. In alkaline solutions of the albumoses the addition of solutions of carmalaun or hæmalaun caused heavy flocculent colored precipitates. It is possible that the precipitate was simply the stain which is insoluble in alkaline solutions, but it is also possible that it was the stain in combination with the albumose. In any case the aluminium salts of carminic acid and hæmatine no longer react toward the albumoses or tissues like acid stains, but like basic stains. This is possibly due to the strong basicity of the aluminium, and its tendency to form double acid salts. It will probably be found, I believe, that the aluminium salts of the acid aniline colors stain like the basic dyes.

#### IV. THE STAINING OF SECTIONS.

The foregoing experiments suggest that the affinity of sections of tissues for stains depends upon reactions similar to the above. So far as I have experimented, the results have fully confirmed this suggestion, but the formation of salts by acids of the tissues with the basic dyes also comes into play. We will consider this first.

A. **The basic dyes in neutral solution.**—The basic dyes in neutral or acid solution will not combine either with albumin or the albumoses. It is clear from this that the affinity shown by chromatin, cartilage, and mucin for such dyes when in neutral solution must depend on something else than the albumin molecule. The suggestion of Miescher and Lilienfeld that the affinity of chromatin for the basic dyes depends on the nucleinic acid indicates the essential cause of the staining reactions of the elements just mentioned. Besides the albumin molecules they contain, mucin, chromatin, and hyaline cartilage have little else in common than the presence in each of organic acids in salt combinations with strong bases. There can be little doubt that the basic dyes in neutral solution will stain any element of the tissue which contains an organic acid in a salt combination with a strong base.

That methyl green in neutral or acid solutions stains those chromatins in which the nucleinic acid exists in a salt form is shown by

its striking affinity for the chromatin of some spermatozoa, thymus gland cells, leucocytes, cells of the spleen, and the red blood corpuscles of birds, and by its slight affinity for the cells of the vertebrate pancreas. In the thymus gland, leucocytes, and red blood corpuscles, Kossel<sup>1</sup> has shown the chromatin to be composed largely of the histon salt of nucleinic acid. In the spermatozoa of the fish and sea-urchin, Miescher,<sup>2</sup> Kossel,<sup>3</sup> and the author<sup>4</sup> have shown the chromatin to be either a histon or protamin salt. In the pancreas on the other hand nucleinic acid exists in a much firmer combination. Lilienfeld's<sup>5</sup> observations on the artificial nucleins confirm this also. He found that the artificial nucleins stained in methyl green so long as they were not saturated with albumin. So soon as the acid became saturated with albumin, the nuclein showed a preponderating attraction for the acid stains. This is strong evidence that the acid stain enters the albumin molecule, while the basic enters the nucleinic acid molecule in these nucleins.

Cytoplasmic bodies with an affinity for basic dyes also indicate that these dyes will stain elements containing the salts of other organic acids. Hyaline cartilage possessing an affinity for such dyes consists, according to Schmiedeberg,<sup>6</sup> largely of the potassium or other salt of the chondroitin-sulphuric acid. Many mucins have the same power of staining in basic stains. The chemistry of mucins is not well known, but many of them, at any rate, react distinctly acid.<sup>7</sup> Many other acids which are possibly present in the cell form insoluble colored salts with the basic dyes. If a basic dye is added to neutral soap solutions a flocculent, colored precipitate consisting probably of the colored salt of palmitic or stearic acid is thrown down. Neutral solutions of thyminic acid, a derivative of nucleinic acid, or of the pseudo-nucleinic acid derived from the yolk of hen's eggs show similar reactions.

These considerations permit us to formulate the following conclusions as to the staining powers of the basic stains. In slightly

<sup>1</sup> See LILIENFELD: *Zeitschr. f. physiol. Chemie.* 1894, xviii, p. 473.

<sup>2</sup> MIESCHER: *Archiv für exper. Pathol. und Pharmacol.*, 1896, xxxvii, p. 100.

<sup>3</sup> KOSSEL: *Zeitschr. f. physiol. Chemie.* 1896, xxii, p. 176.

<sup>4</sup> MATHEWS: *Zeitschr. f. physiol. Chemie.* 1897, xxiii, p. 399.

<sup>5</sup> LILIENFELD: *Archiv für Physiologie*, 1893, p. 391.

<sup>6</sup> SCHMIEDEBERG, O.: *Archiv für exper. Pathol. und Pharmacol.*, 1891, xxviii, p. 355.

<sup>7</sup> HAMMARSTEN: *Zeitschr. f. physiol. Chemie.* 1888, xii, p. 189; also *Lehrbuch der physiologischen Chemie*, ii Aufl., 1896, p. 139.

acid or neutral solutions the basic dyes will stain any element of the tissue which contains an organic acid in a salt combination with a strong base. In no sense are these dyes a test for nucleinic acid or chromatin. All conclusions in regard to the origin of cytological elements from chromatin or their similarity to chromatin based on the staining reaction are hence worth very little. In neutral or acid solutions the basic stains may be used, I believe, as micro-chemical tests of some accuracy for the detection of the salts of organic acids.

**B. The basic stains in acid and alkaline solutions.**—It has been shown above that in acid or neutral solutions the basic stains will not unite with albumin, but in alkaline solution will combine with the albumin molecule. To test the staining reactions of tissues in the basic dyes in the light of this fact, pieces of liver, kidney, and voluntary muscle of the frog were placed in neutral and acidulated ninety-five per cent alcohol. The acidulated alcohol contained one per cent of acetic acid. The tissues were imbedded in paraffine and cut as usual. The fixation was excellent. In staining, all dyes were used in weak aqueous solutions, and the sections were well washed in water before and after immersion in the stain. Sections were left in the dyes from a few seconds to three minutes. If brought into strong solutions of the dyes the tissues imbibe a considerable amount of stain, I presume by a physical process, but this may be entirely removed from the cytoplasm by a comparatively short bath in water or alcohol.

The liver, kidney, and muscle fixed in neutral or acid alcohol give purely chromatin stains with neutral solutions of the dyes vesuvin, methyl green, methylen blue, safranin, toluidin blue, thionin, and dahlia.

In his *Vade-Mecum*, Lee, speaking of methyl green, insists again and again that the stain must be slightly acidulated with acetic acid. With all basic dyes, I have found the result better if a neutral solution is taken, though slight acidification seems to do nothing more than to diminish somewhat the intensity of the stain. In either case a pure chromatin stain is obtained.

When used in alkaline solutions, the basic stains react otherwise. It has been shown that in alkaline solutions the basic dyes combine with albumin. I find that sections of the above mentioned tissue, if immersed for an instant in one-tenth per cent sodium carbonate solution before staining or if stained in solutions of the basic stains made slightly alkaline with sodium carbonate show the cytoplasm deeply

stained, as well as the chromatin. The stain, even in the cytoplasm, is in such firm combination that it is exceedingly difficult, if not impossible, to wash it out. In this manner the cytoplasm of these cells may be stained a bright green with methyl green, brilliant red with safranin, a deep blue with methyl blue or toluidin blue, and purple with thionin. Vesuvin alone seems to be an exception.

These reactions, which are identical with those of the albumoses, show that in alkaline solution many of the basic dyes will combine with the albumin molecule whether in cytoplasm or nucleus. As we have already seen, they probably enter the phenol group of this molecule. The basic dyes in alkaline solution may thus be used for the detection of albumins in the cell, and indeed of albumins possessing a phenol or tyrosin group.

C. **The acid stains.**—The acid stains do not combine with albumin in neutral or alkaline solutions, but only in acid solution. The tissues show the same reaction. Tissues hardened in neutral alcohol will not stain in neutral or alkaline solutions of the acid stains, even such intense stains as acid fuchsin. If brought into concentrated aqueous solutions of these stains, the sections imbibe a certain amount of stain, more or less difficult to remove by washing. If such sections be run rapidly through the alcohols they will appear stained. That the stain in such sections is not in chemical combination is shown by the fact that in dilute solutions of the stains such imbibition is exceedingly slow or wholly lacking, and also by the fact that even after immersion in concentrated staining solutions the stain may be entirely removed by washing some time in water. If, on the other hand, sections of tissues hardened in neutral alcohol are washed before staining with one per cent acetic acid or are brought into acidulated solutions of the acid stains, indigo carmine, carminate of soda, nigrosin, methyl blue, erythrosin, acid green, congo red, orange G., and acid fuchsin, the cytoplasm stains instantly and intensely. The stain cannot be washed out.

The observation that sections of such tissues as liver, kidney, and muscle will not stain in neutral acid fuchsin appears at first glance to be contrary to the common experience that sections will stain in non-acidulated solutions of this color. The contradiction is only apparent. Nearly all fixing reagents are acid, and the free acid undoubtedly combines with the albumin of the protoplasm. Having acid already in combination it is not necessary to acidulate the acid stains, for the sodium of the stain probably unites with the acid derived from

the fixing fluid, and the acid stain replaces this in the albumin molecule. That this is true is shown by the fact that the same tissues hardened in *acid* alcohol stained readily in neutral solutions of the acid stains.

This staining reaction of the tissues with the acid stains, corresponding as it does with the reactions of the albumoses and albumin, enables us to conclude that the acid stains enter into chemical combination with the albumin molecule in protoplasm and probably with an  $\text{NH}_2$  group in that molecule. The acid stains may be used in acid solution on tissue hardened in alcohol or acetic-alcohol, as micro-chemical reagents for the detection of albumin in the cell elements, with the proviso that there may be other unknown basic substances in protoplasm forming similar compounds, and that possibly in some cases the albumin may already be in such combination with other substances that it will not unite with the acid stains.

The observations here recorded by no means elucidate all the phenomena of staining, but, I believe, they indicate one method of attacking the problem. It would be interesting to know what influence the introduction of mercury or other metals and of acids into the albumin molecule may exert on its staining properties. Until this is known the results and conclusions of the present paper cannot be applied to tissues fixed in corrosive sublimate, Hermann's fluid, and many other fixing fluids.

# NOTES ON CETRARIA ISLANDICA (ICELAND MOSS).

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FROM early times lichens have been utilized as articles of diet for man and domestic animals.<sup>1</sup> First among them in importance as a food-stuff is "Iceland moss" (*Cetraria islandica*), which seems to have recommended itself because of its large content of carbohydrate matter, the so-called lichen-starch. In its natural form this lichen contains bitter constituents, and these must be removed by treatment with water or weak alkalies before the material can be made into bread, as has been the custom in some northern countries. Rabbits almost invariably refuse to eat the lichen unless it has been rendered more palatable as described.

With reference to the real dietetic value of *Cetraria islandica*, the following analysis of the commercial material will afford some data.<sup>2</sup>

## *Analysis of Cetraria islandica (dried at 105° C.)*

Total nitrogen . . . . .	0.56 per cent.
Extractive nitrogen . . . . .	0.14 "
"Protein" nitrogen . . . . .	0.32 "
Ether extract <sup>3</sup> . . . . .	1.2 "
Crude fibre . . . . .	5.3 "
Ash . . . . .	2.2 "
Material soluble in 85 per cent alcohol . . . . .	16.1 "
Soluble carbohydrates (as dextrose) . . . . .	43.3 "

After successive treatment with gastric juice and amylolytically and proteolytically active pancreatic juice at 38° C. only 32 per cent of the material used was dissolved. The residue resisting digestion contained practically all the original nitrogen (0.55 per cent) of the lichen.

It will be observed that the quantity of proteids present must be small at most. The bulk of the material is made up of soluble carbohydrates. The latter were early made the subject of chemical in-

<sup>1</sup> Cf. ALBERT SCHNEIDER: A text-book of general lichenology, 1897.

<sup>2</sup> The methods of analysis employed were essentially the same as described by L. B. MENDEL: This journal, 1898, i, p. 226.

<sup>3</sup> This consisted of free fatty acids (0.4 per cent) and saponifiable fat (0.62 per cent).

vestigation. Without attempting to recite the older and somewhat conflicting observations, we may refer to the more recent results of Hönig and St. Schubert.<sup>1</sup> These investigators conclude that extracts of *Cetraria*, obtained with hot water, contain two carbohydrates. The chief one of these, lichenin, forms a difficultly soluble jelly in cold water, an opalescent solution in hot water, is not colored blue by iodine, and does not rotate polarized light; on boiling with dilute acids lichenin yields crystallizable dextrose in addition to dextrins. The second carbohydrate, called lichenin-starch, is regarded by these authors as a soluble modification of ordinary starch. It has also been called isolichenin.<sup>2</sup> Munk<sup>3</sup> states that lichenin is most nearly related chemically to starch, and that it probably undergoes the same fermentative changes in the alimentary canal as are produced by boiling with dilute acids. The following experiments by the writer confirm in part and extend previous observations.

**Lichenin.** — *Preparation.* — The dry assorted Iceland moss was heated in a steam sterilizing apparatus for several hours with a considerable quantity of water, and the extract then filtered on hot water funnels. The cool filtrates deposited a thick jelly which was thrown upon filters and allowed to drain. The gelatinous mass was redissolved in hot water and reprecipitated repeatedly until the cold filtrates as well as the jelly no longer gave any blue coloration with iodine. The gelatinous substance was next treated with warm alcohol until all coloring matter was removed, then extracted with ether and dried. There resulted an almost white, tasteless, odorless powder, soluble in hot water, insoluble in cold water, free from nitrogenous matter, and yielding about one-half per cent of ash.

*Hydration by dilute acid.* — In each trial a weighed quantity of lichenin was boiled for twelve hours with two per cent hydrochloric acid, and the resultant sugar determined in the neutralized fluid by the Allihn gravimetric method. The specific rotation was likewise ascertained and osazones were prepared.

<sup>1</sup> HÖNIG UND ST. SCHUBERT: Sitzungsab. d. k. Akad. d. Wissenschaften zu Wien, 1887, xcvi, 2<sup>te</sup> Abth., p. 685. The older literature is referred to here. Cf. also BEILSTEIN: Handbuch der organ. Chemie, 3<sup>te</sup> Auflage, i, p. 1098.

<sup>2</sup> Cf. BEILSTEIN, *loc. cit.*, p. 1099.

<sup>3</sup> MUNK, J. und C. A. EWALD: Die Ernährung des gesunden und kranken Menschen, 1895, p. 102; also C. VOIT: Die Ernährung. Hermann's Handbuch der Physiologie, 1881, vi, p. 413.



- I. 1.0936 grams lichenin (ash-free) yielded on hydration 1.097 grams dextrose. Assuming a hydration equivalent to that of starch, 1.0936 grams lichenin should yield 1.215 grams sugar.
- II. (a) In a solution of hydration products containing 1.53 per cent sugar (determined as dextrose), in a 200 mm. tube an average of five polariscopic readings gave a rotation of  $+1.6^{\circ}$ . Then  $(\alpha)_D = +52.2^{\circ}$ .  
(b) In a solution containing 0.51 per cent sugar in a 220 mm. tube, an average of six readings gave a rotation of  $+0.6^{\circ}$ . Then  $(\alpha)_D = +53.1^{\circ}$ .  
The specific rotation of dextrose,  $(\alpha)_D = +52.5^{\circ}$ .
- III. The osazones of the sugar formed were prepared with phenylhydrazin in the usual manner, and recrystallized four times from alcohol. M. p.  $199^{\circ} - 201^{\circ}$  C.  
The melting point of phenylglucosazone  $= 204^{\circ}$  C.

The experiments thus indicate an almost complete hydration of lichenin, analogous in its results to the conversion of ordinary starch.

*Action of enzymes and dilute HCl.*—In order to determine the possible fate of ingested lichenin in the alimentary canal, the behavior of the carbohydrate towards the ordinary amylolytic enzymes was reinvestigated. The following typical experiments are selected from the protocols.

- I. A one per cent solution of lichenin in boiling water was prepared and placed in a bath at  $38^{\circ}$  C. Most of the material stays in solution; a portion separates out at this temperature. Saliva was added and the solution was tested for reducing sugars from time to time, with Fehling's solution. No reaction was obtained after *forty-five* minutes. To one portion ordinary starch paste (one per cent) was now added. The solution reached the "achromic point" to iodine solution<sup>1</sup> in *one* minute and sugar was abundantly formed, thus showing that there was nothing present inhibitory to the action of the enzyme. The other portion of the original fluid was unchanged even after several hours.
- II. A very active diastase preparation likewise failed to transform the lichenin to reducing sugar during an hour's action at  $38-40^{\circ}$  C.
- III. To a one per cent lichenin paste was added an amylolytically active pancreatic extract (alcoholic). No sugar was formed, while the unimpaired activity of the enzyme was demonstrated as in Experiment I.
- IV. The ash from one gram of lichenin was added to a small quantity of starch paste. There was no inhibition of the subsequent action of saliva.
- V. A one per cent lichenin paste was treated with saliva for an hour at  $38^{\circ}$  C. No sugar was formed. The solution was then precipitated with alcohol and the precipitate redissolved in water. The action of saliva was again tried, with the usual negative result. These operations were repeated four times with similar effects.

From experiments like the above it must be concluded that the ordinary amylolytic enzymes have no noticeable action on lichenin. Berg<sup>2</sup> is reported to have obtained similar results with saliva, malt diastase, pancreatic extract, and gastric juice. Since it has been shown that cane-sugar is readily inverted in the stomach by the

<sup>1</sup> Cf. GAMGEE: *Physiological chemistry of the animal body*, 1893, ii, p. 57.

<sup>2</sup> BERG: Abstract in *Jahresbericht der Chemie*, 1873, p. 848.

gastric juice<sup>1</sup> and experiments in this laboratory have shown that inulin — likewise resistant to enzymes — is partly transformed to reducing sugar by the action of dilute HCl (0.2–0.4 per cent), the following experiment was tried.

A one per cent lichenin paste was treated with an equal volume of 0.4 per cent HCl and kept at 38° C. for twelve hours. The test for sugar was negative. The mixture was carefully neutralized and treated with amylolytic pancreatic extract. No sugar was formed. Acid of 0.3, 0.4, and 0.5 per cent strength also gave negative results. Glycogen is likewise resistant to the action of these acids at 38° C.

*Feeding experiments.* — In view of the behavior of lichenin already recorded, it seemed desirable to ascertain whether this carbohydrate would give rise to a formation of glycogen in the liver as has been found by Miura<sup>2</sup> to occur after inulin feeding. Miura's experiments were followed as a type and protocols are given below.

Two rabbits, weighing 2.2 and 2.3 kilos respectively, were starved for six days. The control animal (2.3 kilos) was killed and the glycogen content of the liver found by the Brücke-Külz method to be 0.286 gram (0.7 per cent). The other rabbit (2.2 kilos) received ten grams of lichenin, suspended in warm water, in five portions through the stomach sound at intervals of two hours. Twelve hours after the last portion was fed the animal was killed. The glycogen-content of the liver was found to be 0.086 gram (0.25 per cent). Another rabbit of 2 kilos, likewise starved, was fed about eight grams of lichenin in several doses. The animal was accidentally killed immediately after a portion had been fed. The liver did not contain a weighable amount of glycogen.

The writer has not succeeded in finding rabbits that would eat any considerable quantity of the lichen itself, even after extraction with potassium carbonate to remove the bitter taste. Further experiments with larger quantities of lichenin are desirable.

**Isolichenin.** This carbohydrate, to which is due the blue iodine-reaction in the filtrates from the lichenin preparation, has received little investigation.<sup>3</sup> It is in some respects closely related to soluble starch. The amount present in the lichen is decidedly less than the amount of lichenin, and a micro-chemical study shows it to be distributed through the cell walls of both the cortical and medullary portions of the plant. Micro-chemical reactions for cellulose give negative results.

*Preparation.* — The filtrates from the lichenin were concentrated in vacuo at a low temperature (35°–40° C.). If any remaining lichenin settled out on cooling it was filtered off and the solution was treated with several volumes of alcohol. The somewhat gummy precipitate was redissolved in hot water and again cooled. Further traces of lichenin were removed by filtration from the concentrated fluid; the

<sup>1</sup> FERRIS and LUSK: This journal, 1898, i, p. 277.

<sup>2</sup> MIURA, K: Zeitschr. für Biologie, 1895, xxxii, p. 255.

<sup>3</sup> Cf. BERG: *loc. cit.*; HÖNIG und ST. SCHUBERT: *loc. cit.*

isolichenin was precipitated with alcohol, extracted with alcohol and ether, and reduced to an almost white powder, containing 0.4 per cent ash. This preparation dissolves with difficulty in cold water, readily in hot water, from which it does not separate on cooling. With iodine solution it gives a blue coloration.

*Hydration by dilute acid.*—The following data were obtained by the methods already indicated for lichenin.

- I. 1.021 grams isolichenin (ash-free) yielded on hydration 1.125 grams dextrose. Assuming a hydration equivalent to that of starch, the yield of dextrose should have been 1.134 grams.
- II. (a) In a solution of hydration products containing 1.23 per cent sugar (determined as dextrose) in a 200 mm. tube, an average of six polariscopic readings gave a rotation of  $+1.25^{\circ}$ . Then  $(\alpha)_D = +50.8^{\circ}$ .  
(b) In a solution containing 1.13 per cent sugar in a 200 mm. tube, an average of six polariscopic readings gave a rotation of  $+1.17^{\circ}$ . Then  $(\alpha)_D = +51.7^{\circ}$ .  
The specific rotation of dextrose,  $(\alpha)_D = +52.5^{\circ}$ .
- III. The osazones of the sugar formed were prepared and recrystallized four times from alcohol. M. p.  $199^{\circ}$  C. The crystals resemble those of phenylglucosazone in appearance and solubility.

The hydration products of the isolichenin thus correspond closely in behavior with those obtained from the lichenin of the same plant.

*Action of enzymes and dilute HCl.*—Hönig and St. Schubert<sup>1</sup> subjected this carbohydrate to the action of malt diastase at  $60^{\circ}$  C. for several hours. They observed a rapid disappearance of the iodine reaction and formation of dextrin-like substance precipitable by alcohol. From such observations they class isolichenin—their lichen-starch—with soluble starch. The writer has further studied the action of saliva, diastase, and pancreatic extract. Typical experiments are given below.

- I. A one per cent isolichenin solution was treated at  $38^{\circ}$  C. with saliva. The "achromic point" was reached in about one minute, no erythro-dextrin stage being detected. Digestion was continued for an hour. The solution, tested from time to time, gave a slight reduction (with Fehling's solution) which did not increase in amount. Nylander's reagent gave no test for dextrose. The solution was precipitated with alcohol and the filtrate gave no reaction for sugars after removal of the alcohol. The precipitate of dextrin-like substance gave a slight reduction.<sup>2</sup> A flocky blue precipitate was always present in the test. Towards diastase and amylolytic pancreatic extract isolichenin showed similar behavior.
- II. Isolichenin was treated with varying strengths of HCl (0.2–0.5 per cent) at  $38^{\circ}$  C. for twelve hours. No sugar was obtained in any instance.

HÖNIG und ST. SCHUBERT: *loc. cit.*, pp. 694–696.

<sup>2</sup> MUSCULUS and v. MERING (Zeitschr. für physiol. Chemie, 1876, ii, pp. 410–419) obtained from glycogen and starch achroodextrins which likewise slightly reduce Fehling's solution.

The unusual behavior of isolichenin towards amylolytic enzymes — the formation of dextrans without sugars — recalls the formation (from glycogen) of dystropo-dextrin, an achroodextrin resisting the further action of enzymes.<sup>1</sup>

The peculiar carbohydrates of *Cetraria islandica* are doubtless merely types of those occurring in numerous other varieties of this group of plants.

<sup>1</sup> SEEGEN : *Archiv f. d. ges. Physiol.*, 1879, xix, p. 106; TEBB, M. C. : *Journal of physiology*, 1898, xxii, p. 428.

# VARIATIONS IN THE AMYLOLYTIC POWER AND CHEMICAL COMPOSITION OF HUMAN MIXED SALIVA.<sup>1</sup>

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SINCE saliva is the product of secretory glands having their periods of comparative rest and activity, it follows quite naturally that this secretion might be expected to show variations in amylolytic power at different periods of the day: *i.e.*, that the secretion obtained after a period of glandular activity might possess less starch-digesting power than the secretion coming from glands which have been in a state of rest—due mainly to variations in the proportion of active enzyme present. Further, the well-known sensitiveness of the amylolytic enzyme to changes of reaction suggests also the possibility of fluctuations in amylolytic power dependent primarily upon changes in the proportion of alkaline-reacting salts contained in the secretion. In spite of the large amount of work of a chemico-physiological nature done upon saliva, these questions have received very little attention. During the past year, however, Hofbauer<sup>2</sup> in an interesting communication has presented a series of results, bearing on the daily fluctuations in the amylolytic power of saliva, but his observations were limited solely to determination of the starch-digesting power at different periods of the day without regard to any possible relationship between the amylolytic power and the chemical composition of the secretion. His results, however, show clearly that human mixed saliva does fluctuate in amylolytic power throughout the twenty-four hours, and further that the starch-digesting power of the saliva secreted before breakfast, for example, is greater than that of the secretion collected after breakfast. Our results afford distinct confirmation of the general truth of this observation. Hofbauer states in his paper that the only previous work bearing upon

<sup>1</sup> A summary of some of the results contained in this paper was presented at the Meeting of the American Physiological Society in December, 1897, and published in the Proceedings of the Society, this Journal, 1898, ii, p. iii.

<sup>2</sup> HOFBAUER: Archiv f. d. ges. Physiol., 1897, lxx, p. 503.

this subject is that by Chittenden and Ely.<sup>1</sup> The latter work, however, has no bearing whatever upon the question of possible variation in the amylolytic power of the secretion at different periods of the day. Indeed, in the paper in question it is distinctly stated that "the saliva was collected generally an hour or two after breakfast," with the distinct object of avoiding possible variations in composition due to the period of collection. The sole object of that investigation was to ascertain whether there is any connection between possible variations of alkalinity and the amylolytic power of saliva. The results there reported afford no indication whatever of the relative amylolytic action of the secretion for different periods of the day, since the fluids studied were invariably collected at essentially the same hour. It was ascertained, however, that the alkalinity of mixed saliva as measured by titration with a standard acid, using cochineal as an indicator, was fairly constant for a given individual at a given period of the day (9-10 A.M.), while saliva from different individuals may show a constant difference in alkalinity, although in the majority of cases the alkalinity varied only within narrow limits. In amylolytic action, however, there were no corresponding differences; fluctuations were observed, but within too narrow limits to indicate any tangible relation between the two factors.

It has become the custom to assume that the alkalinity of saliva, as indicated by its reaction toward litmus paper, is due more or less to the presence of sodium carbonate. Thus, in the latest text-book of physiology the statement<sup>2</sup> is made that "the alkalinity of saliva depends upon the presence of sodium carbonate. In man and in the dog the percentage of this salt varies from 0.08 to 0.19 per cent." So far as we are aware, however, there is no justification for this statement. In the earlier work from this laboratory<sup>3</sup> it was stated that the average alkalinity for fifty-one samples of human mixed saliva was 0.08 per cent, "expressed in the form of sodium carbonate." Further, in all the tabulated results contained in that paper, the alkalinity, as measured by titration with standard acid in the presence of cochineal as an indicator, was carefully expressed as "equivalent in  $\text{Na}_2\text{CO}_3$ ," this being done to avoid any positive statement as to the exact cause of the alkalinity. Further, in the oft-quoted work of Werther<sup>4</sup> the al-

<sup>1</sup> CHITTENDEN and ELY: American chemical journal, 1883, iv, p. 329.

<sup>2</sup> Text-book of physiology, edited by E. A. Schäfer, 1898, vol. i, p. 504.

<sup>3</sup> CHITTENDEN and ELY: American chemical journal, 1883, iv, p. 333.

<sup>4</sup> WERTHER: Archiv f. d. ges. Physiol., 1886, xxxviii, p. 293.

kalinity of the saliva of the dog was determined by titration with decinormal sulphuric acid with litmus as an indicator: a method which obviously would throw no light upon the cause of the alkalinity. Moreover, in at least some of the tables containing his results the percentage of alkalinity is expressed as "alkalinity calculated as  $\text{Na}_2\text{CO}_3$ ."

Examination of a large number of samples of human mixed saliva obtained from different individuals at different periods of the day convinces us that, under normal conditions at least, human saliva never contains the least trace of sodium carbonate. Toward litmus, lacmoid, etc., human saliva constantly reacts alkaline, but with phenolphthaleïn it invariably shows an acid reaction, and a certain amount of a decinormal alkali solution is required to bring out an alkaline reaction with this indicator. Further, phenolphthaleïn is an extremely sensitive reagent for sodium carbonate; a solution containing 0.001 per cent of sodium carbonate will give a pink color when brought in contact with a solution of phenolphthaleïn. With human saliva, however, we have never obtained any color reaction with phenolphthaleïn whatever; the solution invariably remains colorless, thus proving that the alkalinity indicated by litmus must be due to some acid salt or salts, like the hydrogen alkali phosphates, with possibly some alkali bicarbonate. The submaxillary saliva of the dog, however, obtained by stimulation of the chorda tympani is usually, at least, faintly alkaline to phenolphthaleïn;<sup>1</sup> consequently this fluid may owe its alkalinity in part to sodium carbonate. These facts, which admit of easy confirmation, are worthy of some consideration, since they have an important bearing upon the normal conditions governing enzyme action.

#### I. RELATIVE ALKALINITY AND ACIDITY OF HUMAN SALIVA BEFORE AND AFTER EATING.

In this series of experiments the saliva was collected from one individual, stimulation of the secretion being effected by chewing a small piece of rubber. About 15 c.c. of fluid were collected each time. The portion collected before breakfast was obtained at 7.30 A. M., half an hour before eating, while the portion collected after eating was obtained fifteen minutes after the close of the meal. The alkalinity

<sup>1</sup> CHITTENDEN; *Science*, n. s., 1897, v, p. 902. Also CHITTENDEN, MENDEL, and JACKSON: *This Journal*, 1898, i, p. 174.

was determined by titrating the saliva (5 c.c.) with a decinormal solution of sulphuric acid, using lacmoid as an indicator, while the acidity was determined by the use of a decinormal solution of sodium hydroxide, the indicator being phenolphthaleïn. The alkalinity was calculated in terms of sodium carbonate, and is also expressed as milligrams of  $\text{H}_2\text{SO}_4$  (absolute) required to neutralize 1 gram of saliva. The degree of acidity is expressed as milligrams of NaOH (absolute) required to neutralize 1 gram of saliva. Following are the results obtained: —

Time.	ALKALINITY.		ACIDITY.
	Expressed as $\text{Na}_2\text{CO}_3$ . Per cent.	Milligrams $\text{H}_2\text{SO}_4$ to neutralize 1 gram saliva.	Milligrams NaOH to neutralize 1 gram saliva.
Before Breakfast . . . .	0.163	0.78	0.11
After Breakfast . . . .	0.127	0.61	0.04
Before Breakfast . . . .	0.193	0.93	0.06
After Breakfast . . . .	0.130	0.64	0.08
Before Breakfast . . . .	0.142	0.69	0.06
After Breakfast . . . .	0.122	0.59	0.06
Before Breakfast . . . .	0.132	0.64	0.10
After Breakfast . . . .	0.132	0.64	0.08
Before Breakfast . . . .	0.173	0.83	0.08
After Breakfast . . . .	0.127	0.61	0.04
Before Breakfast . . . .	0.148	0.71	0.11
After Breakfast . . . .	0.132	0.64	0.08
Before Breakfast . . . .	0.168	0.81	....
After Breakfast . . . .	0.127	0.61	....
Before Breakfast . . . .	0.122	0.59	0.11
After Breakfast . . . .	0.122	0.59	0.08
Before Breakfast . . . .	0.148	0.71	....
After Breakfast . . . .	0.137	0.66	....
Before Dinner . . . .	0.132	0.64	0.08
After Dinner . . . .	0.142	0.69	0.02
Before Dinner . . . .	0.168	0.81	0.08
After Dinner . . . .	0.158	0.76	0.08
Before Dinner . . . .	0.153	0.73	0.06
After Dinner . . . .	0.158	0.76	0.02



A glance at these results shows that the alkalinity of saliva, as indicated by lacmoid, is noticeably greater in most cases in the fluid secreted after a night's rest, before breakfast, than in the secretion obtained after the glandular activity induced by the morning meal. Before and after dinner, however (1 P. M.), this distinction is less conspicuous. It is also interesting to note that the average alkalinity, expressed in terms of sodium carbonate, is somewhat higher with lacmoid as an indicator than with litmus or cochineal; a fact which would be expected in view of the presence of the hydrogen alkali phosphates contained in the fluid. It is likewise to be seen that the average acidity as indicated by phenolphthalein, though less conspicuous, is also inclined to diminish after eating.

## II. RELATIVE ALKALINITY AND AMYLOLYTIC POWER OF HUMAN SALIVA BEFORE AND AFTER EATING.

In this series of experiments the main object was to ascertain whether there are noticeable variations in the amylolytic power of saliva before and after eating and whether such variations, if existent, run parallel with variations in the alkalinity. As in the previous experiments, the saliva was collected by chewing a small piece of rubber.

Amylolytic power was determined as follows; 5 c.c. of the filtered saliva were diluted with distilled water to 50 c.c.; 10 c.c. of the diluted fluid were then added to 1 gram of pure arrowroot starch made into a paste with 90 c.c. of water, and the mixture kept at 38° C. for half an hour. Amylolysis was then stopped by boiling the fluid, after which the solution, when cool, was made up to 150 c.c. with water and the reducing sugar determined by the Allihn Method, using 25 c.c. of the sugar-containing solution. The results are expressed as milligrams of maltose formed from 1 gram of starch by 1 c.c. of saliva. The data obtained are given on the next page.

From these results it would appear that saliva secreted after a period of glandular inactivity, as before breakfast, is ordinarily possessed of greater amylolytic power than the secretion obtained after eating; results which accord closely with Hofbauer's observations. Before and after dinner (1 P. M.), however, the difference in amylolytic power is less pronounced; a fact which might be expected in view of the short period for recuperation between the breakfast and dinner and because of the more or less constant stimulation of the salivary glands during the waking hours. Further, we see in these results a suggestion of some degree of relationship between the percentage of

Collector.	Time.	ALKALINITY.		AMYLOLYTIC POWER. Milligrams Maltose formed by 1 cc. saliva.
		Expressed as $\text{Na}_2\text{CO}_3$ . Per cent.	Milligrams $\text{H}_2\text{SO}_4$ to neutralize 1 gram saliva.	
R.	Before Breakfast	0.173	0.83	523.4
	After Breakfast	0.127	0.61	511.8
R.	Before Breakfast	0.168	0.81	630.6
	After Breakfast	0.127	0.61	583.8
R.	Before Breakfast	0.148	0.71	562.2
	After Breakfast	0.132	0.64	485.4
R.	Before Breakfast	0.122	0.59	620.4
	After Breakfast	0.122	0.59	534.6
R.	Before Breakfast	0.148	0.71	549.0
	After Breakfast	0.137	0.66	510.5
J.	Before Breakfast	....	....	209.4
	After Breakfast	....	....	224.4
M.	Before Breakfast	0.117	0.56	585.0
	After Breakfast	0.102	0.49	468.6
M.	Before Breakfast	....	....	621.0
	After Breakfast	....	....	537.6
R.	Before Dinner	0.153	0.73	549.6
	After Dinner	0.158	0.76	536.4
R.	Before Dinner	0.142	0.69	582.0
	After Dinner	0.142	0.69	564.6
R.	Before Dinner	....	....	570.0
	After Dinner	....	....	562.8
R.	Before Dinner	0.163	0.78	599.8
	After Dinner	0.158	0.76	606.6
R.	Before Dinner	....	....	594.0
	After Dinner	....	....	547.2

alkaline salts contained in the saliva and its amylolytic power. Before breakfast, for example, the content of alkaline salts and the starch-digesting power of the secretion are greater than in the fluid secreted after glandular activity. At first glance, then, it might seem that the variations in amylolytic action noticed above are due to changes in the proportion of alkaline salts. The objection to this view, however, is that it associates the higher degree of amylolytic power with the

higher percentage of alkalinity, whereas numerous trustworthy experiments tend to show that saliva manifests its highest degree of digestive power in a perfectly neutral fluid.<sup>1</sup> Consequently, if the above variations in amylolytic action are primarily due to changes in the proportion of alkaline-reacting salts, then the higher degree of amylolysis should be connected with the lower degree of alkalinity. As the reverse is true, the more plausible and natural explanation of the results is that the higher degree of amylolysis is connected primarily with the presence of larger amounts of the amylolytic enzyme, and as this is presumably connected with the outpouring of a more concentrated secretion a corresponding increase in alkaline-reacting salts might naturally be expected. Further, in harmony with the latter view it is to be noticed that the secretions obtained before and after breakfast fail to show any close parallelism between the variations in amylolytic power and variations in alkalinity. Thus, the most marked differences in digestive power are frequently seen with salivas which show only a slight difference in alkalinity, and on the other hand marked differences in alkalinity may be associated with minor differences in amylolytic power.

### III. ALKALINITY, AMYLOLYTIC POWER, AND COMPOSITION OF HUMAN SALIVA BEFORE AND AFTER EATING.

In view of the preceding results, the following set of experiments was tried in which, in addition to alkalinity and amylolytic power, the proportion of dry solids and inorganic salts of the saliva was likewise determined. The dry solids were determined by simply drying a weighed amount of the filtered saliva—usually five grams—on a water-bath and heating at 105° C. in an air-bath until of constant weight. The inorganic salts were then determined by careful ignition of the residue. In some of the following experiments relative amylolytic action was determined by Robert's<sup>2</sup> method, the method being based on the different lengths of time which solutions of different amylolytic power require to digest a certain amount of starch paste to the achromic point. The results obtained by this method are expressed in minutes; *i. e.*, the number of minutes which elapse from the time the diluted saliva is added to the starch paste until the appearance of the achromic point.

<sup>1</sup> LANGLEY and EVES: *Journal of physiology*, 1883, iv, p. 18. CHITTENDEN and SMITH: *Studies in physiol. chemistry*, Yale University, 1885, i, p. 8.

<sup>2</sup> See Gamgee's *Physiological chemistry of the animal body*, vol. 2, p. 56.

Following are the results obtained : —

Collector.	Time.	Vol. c.c.	ALKALINITY.		AMYLOLYTIC POWER. Mg. maltose formed by 1 c.c. saliva.	Total solids. Per cent.	Organic matter. Per cent.	Inorganic salts. Per cent.
			AsNa <sub>2</sub> CO <sub>3</sub> Per cent.	Mg.H <sub>2</sub> SO <sub>4</sub> to neutral- ize 1 gram saliva.				
	Breakfast.							
R.	Before	30	0.158	0.76	649.2	1.02	0.77	0.24
	After	25	0.122	0.59	601.2	0.51	0.33	0.17
R.	Before	25	0.163	0.78	651.0	0.86	0.58	0.28
	After	40	0.112	0.55	615.6	0.51	0.30	0.21
R.	Before	20	0.122	0.59	467.4	0.44	0.23	0.22
	After	25	0.102	0.49	491.4	0.40	0.19	0.21
D.	Before	20	0.081	0.39	43.0 <sup>1</sup>	0.37	0.21	0.16
	After	20	0.096	0.46	50.0	0.39	0.24	0.15
A.	Before	50	0.153	0.73	12.0	0.45	0.30	0.15
	After	40	0.158	0.76	15.0	0.53	0.34	0.19
B.	Before	40	0.137	0.66	13.0	0.32	0.15	0.17
	After	30	0.132	0.64	8.0	0.37	0.21	0.16
<sup>1</sup> In this and the two following experiments amylolytic power was determined by Robert's method.								

In these results we have a suggestion of the same general tendency toward decrease of amylolytic power and lowered content of alkaline salts in the saliva secreted after the morning meal, while as accompanying results we see corresponding fluctuations (although not in all cases) in the proportion of total solids, organic matter, and inorganic salts, thus bearing out the view that the variations in amylolytic power are connected mainly with changes in the general concentration of the secretion. At the same time it is to be observed that the above differences in composition and amylolytic power are much more marked with the individual R than with A, B, and D. In fact, with the latter three individuals there is very little difference in composition in the saliva before and after the morning meal, and further in the third experiment with R the amylolytic power *after* the meal is greater than that of the saliva secreted before eating. These results have led to another series of experiments having in view especially the determination of the fluctuations in the character of the saliva throughout the day.

IV. VARIATIONS IN THE COMPOSITION AND AMYLOLYTIC POWER OF HUMAN SALIVA THROUGHOUT THE DAY.

In the first series of experiments under this head the saliva studied was collected by chewing a piece of rubber. The mid-day dinner was omitted; breakfast, however, was taken at 7.50 A. M. and supper at 6.40 P. M. Samples of saliva were analyzed every hour or two throughout the day. Following are the results obtained: —

Date.	Time.	Volume saliva. c.c.	Alkalinity calculated as $\text{Na}_2\text{CO}_3$ . Per cent.	Amylolytic power. Milligrams maltose.	Total solids. Per cent.	Organic matter. Per cent.	Inorganic salts. Per cent.
Jan. 20	<b>A.M.</b> 7.15 to 7.30	21	0.112	574.2	0.59	0.29	0.30
" 20	7.50 to 8.15, Breakfast						
" 20	8.40 to 8.55	25	0.091	469.2	0.41	0.18	0.23
" 20	10.00 to 10.15	29	0.102	544.8	0.44	0.24	0.20
" 20	11.00 to 11.18	23	0.102	517.2	0.40	0.23	0.17
" 20	<b>P.M.</b> 12.00 to 12.13	21	0.132	183.6	0.39	0.16	0.23
" 20	12.45 to 12.55	19	0.112	280.8	0.40	0.20	0.20
" 20	2.00 to 2.15	21	0.112	270.6	0.37	0.16	0.21
" 20	3.00 to 3.12	21	0.132	217.8	0.44	0.26	0.18
" 20	4.00 to 4.13	22	0.132	382.8	0.47	0.27	0.20
" 20	5.00 to 5.14	23	0.153	575.4	0.54	0.29	0.25
" 20	7.00 to 7.25, Supper						
" 20	8.30 to 8.45	29	0.153	513.0	0.49	0.25	0.24
" 20	10.40 to 10.55	28	0.163	459.6	0.55	0.38	0.17

Here, as in the preceding experiments, there is noticeable the same diminution of amylolytic power, alkalinity, and content of solid matter, etc., in the mixed saliva secreted directly after the morning meal. Of special significance, however, is the marked variation in the values throughout the day, thereby suggesting the existence of a normal curve of secretion. Thus, after the morning meal the saliva shows the effect of the stimulation by its lower content of solids, etc. Soon after, however, there is an upward tendency; the curve rises, and amylolytic power is increased as well as the alkalinity, together with the total solids and organic matter. The inorganic salts, on the

other hand, still remain low. Towards noon time, amylolytic power sinks very greatly, and there is a corresponding drop in the proportion of organic solids, although the alkalinity and inorganic salts still remain fairly high. After this, amylolytic power gradually rises, reaching the maximum again at 5 P. M. with a corresponding rise in alkalinity, total solids, etc. Supper at 7 P. M. apparently causes a slight fall in amylolytic power, together with a fall in the solid matter secreted. At 10.40 P. M. amylolytic power shows a still greater fall, although alkalinity and solid matter are increased in amount.

How far are the preceding variations in the secreted saliva due to the combined influence of taking food and the mechanical stimulation incidental to mastication of the rubber, and how far to a natural variation in the composition of the secretion? This question we have endeavored to answer by noting the variations in the saliva on a day when food was abstained from, and by collecting the saliva without movement of the jaws. This was accomplished by simply resting the head on the hands, with the mouth downwards, and allowing the saliva to drip into a beaker without any unnecessary movement.<sup>1</sup> In this way 15–20 c.c. of saliva were collected in half an hour.

Following are the results obtained: —

Date.	Time.	Volume saliva. c.c.	Alkalinity calculated as Na <sub>2</sub> CO <sub>3</sub> . Per cent.	Amylolytic power. Milligrams maltose.	Total solids. Percent.	Organic matter. Per cent.	In- organic salts. Per cent.
Jan. 26	Midnight 11.45 to 12.15	15	0.081	490.4	0.38	0.23	0.15
" 27	A.M. 6.40 to 7.30	13	0.088	572.4	0.63 <sup>1</sup>	0.47	0.16
" 27	9.30 to 10.00	15	0.092	558.6	....	....	....
" 27	11.00 to 11.30	20	0.071	381.0	0.33	0.18	0.15
" 27	P.M. 12.25 to 12.50	17	0.102	441.0	0.37	0.19	0.18
" 27	2.15 to 2.45	16	0.092	347.4	0.32	0.20	0.12
" 27	4.00 to 4.30	19	0.091	416.4	0.35	0.19	0.16
" 27	5.15 to 5.50	18	0.102	423.0	0.35	0.19	0.16
" 27	7.00 to 7.25, Supper						
" 27	8.30 to 9.00	20	0.102	403.2	0.43	0.28	0.15

<sup>1</sup> This result is of somewhat questionable accuracy, having been obtained with a very small amount of saliva.

<sup>1</sup> See HOFBAUER: *loc. cit.*, p. 503.

A study of these results shows clearly that when the stimulating influences of food and mastication are withdrawn, conspicuous alterations in the composition and physiological action of the saliva are still found, as though there might be a normal curve independent of the fluctuations induced by stimuli. Thus at 11.30 A. M. there is seen the same fall in amylolytic power that was so conspicuous in the preceding experiment. Further, the saliva secreted at 2.15 P. M. shows a diminution in amylolytic power, as noticeable as the diminution frequently observed after a hearty meal. It is thus quite evident that in the absence of food and other stimulation hourly changes in the amylolytic power of mixed saliva may occur just as marked as those noticed in the saliva secreted before and after breakfast. Variations in alkalinity, total solids, etc. are not so prominent. It is to be noticed, however, from the last series of experiments, that in the absence of breakfast there is no great variation in the amylolytic power of the saliva secreted between 6.40 and 11.00 A. M.; consequently we may accept the conclusion, justified by the results of most of our experiments, that the taking of food, as at breakfast, tends to lower the starch-digesting power of the saliva secreted some time thereafter. This being so it seems probable that other forms of stimulation may likewise give rise to a change in the composition and physiological action of mixed saliva.

#### V. INFLUENCE OF VARIOUS STIMULI ON THE COMPOSITION AND AMYLOLYTIC POWER OF HUMAN SALIVA.

In this series of experiments the attempt was made to ascertain how far the character of the stimulus modifies the properties of mixed saliva. The special stimuli employed were ether, alcohol, whiskey, and gin. The first two were taken into the mouth in the form of vapor, and the saliva allowed to trickle from the mouth without motion of the jaws, the fluid so obtained being compared with saliva resulting from the mechanical stimulation produced by chewing a piece of rubber. With whiskey and gin, the mouth was well rinsed with the fluid and the saliva collected by allowing it to flow from the corner of the mouth. The control experiments with water were made in the same way; *i.e.*, the mouth was rinsed with water and the saliva allowed to trickle forth. Finally, for the sake of comparison and to ascertain how far two samples of saliva obtained at such close intervals, under similar forms of stimulation, differ from

each other, four control experiments were tried with water and rubber alone.

Following are the results obtained: —

Date.	Time.	Stimulus.	Volume saliva c.c.	Alkalinity calculated as $\text{Na}_2\text{CO}_3$ . Per cent.	Amylolytic power. Milligrams maltose.	Total solids. Per cent.	Organic matter. Per cent.	Inor- ganic salts. Per cent.
Dec. 3	A.M. 11.05-11.30	Rubber	40	0.168	582.6	0.63	0.31	0.32
	11.30-11.50	Ether	30	0.204	624.6	0.76	0.54	0.22
" 9	9.50-10.10	Rubber	30	....	562.8	0.54	0.30	0.24
	10.10-10.30	Ether	25	....	498.6	0.54	0.29	0.31
" 13	11.40-12.00	Rubber	40	0.122	472.2	0.41	0.21	0.20
	P.M. 12.00-12.35	Alcohol	28	0.132	510.6	0.43	0.19	0.24
" 14	A.M. 10.00-10.30	Water	30	0.061	473.4	0.32	0.19	0.13
	10.30-11.00	Whiskey	35	0.102	485.4	0.42	0.29	0.13
" 16	10.15-10.40	Water	23	0.071	483.6	0.34	0.20	0.14
	10.45-11.20	Gin	24	0.102	642.0	0.53	0.36	0.17
" 17	10.20-10.35	Ether	27	0.122	586.2	0.32	0.16	0.16
	10.45-10.55	Rubber	28	0.183	577.2	0.52	0.24	0.28
" 20	11.15-11.45	Water	24	0.071	606.6	0.68	0.55	0.13
	P.M. 12.15-12.45	Water	24	0.102	564.0	0.38	0.27	0.11
Jan. 11	3.03- 3.35	Water	26	0.053	436.8	0.30	0.16	0.14
	4.05- 4.40	Water	30	0.081	532.2	0.35	0.21	0.14
" 13	A.M. 11.25-11.40	Rubber	30	0.153	571.8	0.49	0.26	0.23
	P.M. 12.10-12.26	Rubber	30	0.261	550.8	0.47	0.24	0.23
" 14	A.M. 10.38-10.58	Rubber	34	0.132	577.8	0.50	0.27	0.23
	11.30-11.45	Rubber	32	0.142	594.6	0.51	0.26	0.25

A glance through these results shows at once certain marked differences in the character of the saliva obtained under the different conditions specified. Thus, saliva which flows from the mouth after the latter has been rinsed once with water invariably shows a lower degree of alkalinity, and generally contains a smaller percentage of solid matter, than the secretion obtained by the other methods. In amylolytic power, however, there is great variation; some samples showing a relatively strong amylolytic action, while others with essentially the same degree of alkalinity are much weaker in their



starch-digesting power. Simple mastication of rubber has a marked influence in raising the content of alkaline salts in the saliva, as well as the total inorganic constituents, and there is a tendency toward increase in amylolytic power although the latter is not constant.

As to the influence of alcohol, ether, gin, and whiskey, there is, we think, no question that these agents taken into the mouth change the character of the secretion, increasing its alkalinity, amylolytic power, and content of solid matter. This is certainly true if the secretion so obtained is compared with the saliva flowing from the mouth without stimulation of any kind. Saliva, however, secreted under the stimulation produced by chewing rubber, is, as we have seen, comparatively concentrated, and the difference between the secretion resulting from that method and the fluid coming from ether, alcohol, and other like forms of excitation, without mechanical stimulation, is not so decisive in the above experiments as to make the matter quite clear, especially in view of the fact that two portions of saliva obtained one after the other, by the same method of stimulation, are liable to show marked differences in composition and reaction. Particularly noteworthy is the fact that of two portions of saliva collected one after the other by mechanical stimulation (chewing rubber) or by simply allowing the saliva to flow from the mouth after once rinsing the latter with water, the latter portion of saliva is, as a rule, more concentrated and possessed of higher amylolytic power than the portion first secreted. It is thus obvious that great care must be exercised in drawing deductions from the composition and amylolytic action of mixed saliva when the latter is so prone to vary under what seem to be essentially the same forms of stimulation. It is furthermore equally obvious that the possible causes to which the above variations may be attributed are many, since there are involved three distinct sets of glands in addition to the buccal glands of the mouth cavity. Hence, increase or decrease in amylolytic power, as well as in the general concentration of the secretion, may involve simply an alteration in the relative activity of the individual glands and not be connected primarily with any specific stimulation of metabolic or secretory activity.

However this may be, it is quite clear that the natural variations in the character of the mixed saliva, indicated by the results of the last four experiments of the above series, render it necessary to use great

caution in arranging the conditions under which the experiments are tried. We have therefore repeated the above experiments, choosing for the collection of the saliva a time of day when we have found the mixed saliva most constant in composition; viz., between 9.30 and 10.30 A.M. To be sure, there are variations in the composition and starch-digesting power of successive portions of saliva collected by the same method at this period, but they are relatively small; quite small, indeed, as compared with the variations liable to occur at other periods of the day. The truth of this statement is illustrated by the two following experiments, in which the saliva was collected without stimulation, simply allowing it to flow from the mouth.

Date.	Time.	Volume saliva c.c.	Alkalinity as $\text{Na}_2\text{CO}_3$ . Per cent.	Amylolytic Power. Milligrams maltose.	Total Solids. Percent.	Organic constitu- ents. Percent.	In- organic salts. Percent.
Feb. 3	A.M. 9.32 to 10.06	21.0	0.0816	569.4	0.50	0.31	0.19
" 3	10.15 to 10.42	22.0	0.0918	549.0	0.46	0.29	0.17
" 3	P.M. 5.00 to 5.20	19.5	0.0918	573.6	0.49	0.31	0.18
" 3	5.27 to 5.50	17.0	0.1122	613.8	0.68	0.51	0.17

Thus, the two portions collected between 9.32 and 10.42 A.M. are essentially alike, while the two fractions secreted between 5.00 and 5.50 P.M., all without stimulation, are more dissimilar. Adopting the morning hour as the better time for collection, experiments were tried with alcohol, ether, chloroform, whiskey, and gin, comparing in each case the saliva obtained under their influence with the secretion coming without stimulation of any kind. The exact method pursued in the case of the control, *i. e.*, with water, was to rinse the mouth once with distilled water after which the saliva was simply allowed to drop from the mouth into a beaker. With ether and chloroform the mouth was filled once with the vapor and the saliva then allowed to flow spontaneously into a receptacle without any motion of the jaws. With the alcohol, gin, and whiskey 10 c.c. of the fluid were taken into the mouth, held a moment, and then ejected, after which the saliva was collected as in the other cases. Lastly, an experiment was tried (Feb. 15) by chewing rubber as a stimulant, and comparing the

saliva so obtained with a control secreted without stimulation. Following are the results obtained:

Date.	Time.	Stimulus.	Vol. saliva c.c.	Alkalinity as $\text{Na}_2\text{CO}_3$ Per cent.	Amylolytic Power. Milligrams maltose.	Total solids. Per cent.	Organic constituents. Per cent.	Inorganic salts. Per cent.
Feb. 7	A.M.							
	10.05-10.32	Water	18.0	0.0714	480.6	0.42	0.22	0.20
	10.37-10.56	40% Alcohol	18.0	0.1122	514.2	0.43	0.26	0.17
" 8	9.37-10.05	Water	18.0	0.0612	566.4	0.42	0.25	0.17
	10.11-10.32	Ether	18.0	0.1122	558.6	0.54	0.29	0.25
" 10	9.53-10.18	Water	17.5	0.0816	604.2	0.51	0.33	0.18
	10.27-10.47	Chloroform	17.0	0.0714	644.4	0.69	0.48	0.21
" 11	9.40-10.07	Water	17.0	0.0714	493.3	0.39	0.25	0.14
	10.14-10.36	Whiskey	17.0	0.1020	547.8	0.50	0.31	0.19
" 15	9.52-10.16	Water	16.5	0.0816	541.2	0.38	0.21	0.17
	10.21-10.27	Rubber	17.0	0.1530	577.2	0.58	0.26	0.32
" 18	9.33-10.03	Water	17.0	0.0714	584.4	0.49	0.33	0.16
	10.10-10.34	Gin	19.0	0.1020	610.2	0.57	0.39	0.18
" 23	9.26- 9.51	Water	17.0	0.0714	429.6	0.30	0.18	0.12
	10.01-10.24	Water	17.5	0.0714	423.0	0.31	0.18	0.13

From these results it would seem quite clear that the several agents employed, with the exception of chloroform, give rise to a marked increase in the content of alkaline-reacting salts in mixed saliva. Mechanical stimulation, as by chewing rubber, however, is even more effective than the chemical stimuli employed, although it must not be overlooked that in the above experiments the action of alcohol, ether, whiskey, etc., is necessarily of short duration. Further, there is evidence in most of the results of an increase in amylolytic power, as well as in the content of solid matter under the influence of the stimuli. It is thus safe to assert that alcohol and alcoholic fluids not only stimulate the flow of saliva, but that they also tend to increase the concentration and amylolytic power of human mixed saliva,—results which are in close accord with the action of these fluids upon the secretion of the sub-maxillary saliva of the dog.<sup>1</sup> Further, simple mechanical stimulation, as mastication, may also

<sup>1</sup> See CHITTENDEN, MENDEL, and JACKSON: This journal, 1898, i, p. 167.

increase the amylolytic power of mixed saliva. Lastly, it should be mentioned that the saliva resulting from the above forms of stimulation, excepting mechanical stimulation, is much more viscid than the fluid secreted spontaneously, evidently from a higher content of mucin.

#### SUMMARY.

Human mixed saliva contains normally no sodium carbonate whatever; the alkalinity indicated by litmus, lacmoid, etc., is due to hydrogen alkali phosphates, with possibly some alkali bicarbonate. Mixed saliva invariably reacts acid to phenolphthaleïn.

The alkalinity of mixed saliva, as indicated by lacmoid, is greater before breakfast than after the morning meal; a conclusion which stands in direct opposition to the statement frequently made that "the alkalinity (of mixed saliva) is least when fasting, as in the morning before breakfast, and reaches its maximum with the height of secretion during or immediately after eating."<sup>1</sup>

Saliva secreted after a period of glandular inactivity, as before breakfast, manifests greater amylolytic power than the secretion obtained after eating, as observed by Hofbauer. Corresponding with this increase in amylolytic power occurs an increase in the proportion of alkaline-reacting salts, but the increased amyolysis is due primarily to an increase in the amount of active enzyme contained in the saliva.

Mixed saliva, whether collected by mechanical stimulation or collected without effort, shows a natural tendency to vary both in composition and in amylolytic power throughout the twenty-four hours, and apparently independent of the taking of food. Between 7.00 and 11.00 A.M., however, in the absence of food the secretion is remarkably constant.

Mechanical stimulation, as chewing a tasteless substance, and alcohol, ether, gin, whiskey, etc., taken into the mouth, all lead to the outpouring of a secretion richer in alkaline-reacting salts and in amylolytic power than the secretion coming without stimulation.

Mixed saliva resulting from stimulation with ether, alcohol, etc., contains a much larger proportion of mucin than the secretion coming without stimulation, being noticeably thick and viscid. This quality is not apparent in the saliva resulting from mechanical stimulation.

<sup>1</sup> Text-book of physiology, edited by E. A. SCHÄFER, 1898, i, p. 344.

## THE VENOMOTOR NERVES OF THE HIND LIMB.

By F. W. BANCROFT.

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ALTHOUGH several investigations of the venomotor nerves of other regions, particularly the portal vein, have been published, the literature of the venomotor nerves of the hind limb is limited to the single paper of Thompson.<sup>1</sup> On stimulating the sciatic nerve or the spinal cord of four dogs, Thompson observed that the superficial veins of the hind limb were constricted. The constriction did not extend throughout the vein exposed, but was limited to short sections, between which the diameter remained unchanged. The same result was obtained in four of the five rabbits used.

In my experiments rabbits and cats were employed. The cat is much more satisfactory than the rabbit. The sciatic nerve was severed under ether and the peripheral end stimulated with a weak interrupted induction current while the superficial veins on the outside of the hind limbs were examined. Contractions of the skeletal muscles were prevented by curare. At first the aorta was ligated before stimulation — to exclude the possibility of a decrease in the diameter of the observed vein in consequence of constriction of the arteries of the limb.<sup>2</sup> The veins were kept covered by the skin when not actually under examination. Closing the aorta did not cause any marked decrease in the diameter of the vein, but merely a flattening and general flabbiness throughout its extent. The stimulation on the other hand caused a marked constriction, which was quite irregularly localized. Usually the constricted segments were short, but occasionally a piece ten or twenty millimetres in length would contract uniformly. After a brief exposure to the air the contractions were more variable than at first, parts that had formerly contracted now often failing.

More uniform results were gained when the vein was kept from drying and cooling by irrigation with warm normal saline solution. A flap of skin was raised to form a small reservoir for the saline

<sup>1</sup> THOMPSON: *Archiv für Physiologie*, 1893, p. 102.

<sup>2</sup> The closing of the aorta was omitted in the later experiments on cats, as it was found to make no essential difference in the result.

solution, in which the vein lay exposed. The part contracted by stimulation was now much longer. Thus in the rabbit the vein occasionally contracted uniformly over a length of seventy millimetres, and contractions of thirty to forty millimetres were the rule. In the cat, the length usually contracting was even greater. But even with the warm saline solution the phenomena were not constant, some parts tiring rapidly and failing to constrict after several stimulations, while others continued with hardly diminished vigor. The position of the latter was usually the same in different individuals. A part of the vein about twenty millimetres in length — just before the vein leaves the surface and passes between the underlying muscles to enter the pelvic cavity — never contracted in any of the rabbits. This part is probably supplied with constrictor fibres through some nerve other than the sciatic, or else the constrictor fibres leave the sciatic on the central side of the point stimulated.

The character of the contraction admits no doubt that it is caused by the vasomotor nervous mechanism. Usually the change in size is considerable, and there is no difficulty in determining whether the vein is constricted or not. Simple inspection of the vein, however, cannot determine with certainty the smaller changes of calibre or the exact time of their beginning. At first an interrupted current that is just distinct on the tongue will usually decrease the diameter of the vein one-third, and sometimes will obliterate the lumen so that no blood can be seen; but the venomotor apparatus is soon tired and then a stronger stimulus is necessary to produce a decided contraction. The latent period is quite long, varying from about ten to twenty or even to thirty seconds. Stronger and more frequent induction shocks decrease the latent period and increase the constriction.

Having determined the presence of venomotor fibres in the sciatic nerve, the next step was to trace them from the spinal cord. For this purpose only cats were used, as they endure the operation much better than rabbits. The animals were anæsthetized with ether during the preparation of the nerves and the vein. The stimulation, which was limited to the peripheral segment of the nerves, was done under curare. The characteristic changes in the vein occurred whether the animal was completely or incompletely under the influence of the drug, but in order to make sure that no activity of the voluntary muscles was responsible for the constriction no results were considered as final unless the curarization was complete.

The part of the hind extremities the veins of which have been particularly examined in determining the course of the venomotor fibres is the lateral surface of the crus, and the pes. All the veins in the former and most of those in the latter region have been seen to contract at one time or another. Since the diagonal vein in the lower part of the crus was the most reliable, the majority of the observations were made in its immediate neighborhood so as to have smaller and fewer cuts in the skin. The veins of the thigh and the medial surface of the crus are apparently less sensitive, for I have as yet not seen them contract; but the number of observations on these veins was small.

To determine the origin of the venomotor fibres from the spinal cord the roots were cut and the peripheral segments stimulated within the vertebral canal. In order to facilitate the operation both the anterior and the posterior roots were tied together outside the dura mater. The cord was removed in the region stimulated so that the possibility of leakage of the current to the cord was excluded. Negative results were never accepted as evidence of the absence of venomotor fibres in the nerve stimulated unless the stimulation of some other spinal nerve, or of the sciatic, gave constriction of the vein and thus proved that the vasomotor apparatus was in working order.

The venomotor fibres to the hind limb, as may be seen from Table I, may be demonstrated in the I to IV lumbar nerves, but in no case were they found in more than three of these in any one animal, and in about half the cases they were found in only two of the nerves. The greatest constriction in every animal but one followed stimulation of the III lumbar nerve. In this exceptional case, the IV lumbar nerve was the most efficient. As this was the only instance in which the lumbo-sacral plexus was of Langley's posterior type<sup>1</sup> it may be that in this type the IV nerve is commonly the most effective. The nerves that produced the most vigorous contraction also influenced a greater length of the vein. There was no definite localization of the area supplied by one nerve, such as was observed later when the gray rami communicantes were stimulated.

From the spinal cord the venomotor fibres enter the sympathetic system. It is *a priori* probable that their course is through the white rami of the spinal nerves, the stimulation of which produces contraction. The highest part of the sympathetic that has given any contraction of the vein is immediately below the III lumbar ganglion.

<sup>1</sup> LANGLEY: Journal of physiology, 1894, xvii, p. 296.

TABLE I.

VENOMOTOR FIBRES IN THE SPINAL NERVES.													
Number of Experiment.	Tho-racic.	Lumbar.							Sacral.				Character of Plexus.
	xiii	i	ii	iii	iv	v	vi	vii	i	ii	iii	iv	
IV	..	..	..	..	..	o	..	..	..	..	..	..	....
VI	..	..	..	..	..	o	o	..	..	..	..	..	....
VII	..	..	..	..	..	..	o	o	..	..	..	..	....
VIII	..	..	..	..	..	..	..	..	o	o	..	..	....
IX	..	c <sup>2</sup>	c <sup>2</sup>	c <sup>1</sup>	..	..	..	..	..	..	..	..	....
X	..	..	?	c	?	..	..	..	..	..	..	..	....
XIII	..	..	..	c <sup>2</sup>	c <sup>1</sup>	o	..	..	..	..	..	..	Post. b
XIV	..	..	..	..	..	..	..	o	..	o	o	o	Post. b
XV	o	o	o	c <sup>1</sup>	c <sup>2</sup>	o	..	..	..	..	..	..	Ant.
XVI	..	o	c <sup>3</sup>	c <sup>1</sup>	c <sup>2</sup>	o	..	..	..	..	..	..	Ant.
XVIII	..	o	c <sup>2</sup>	c <sup>1</sup>	o	..	..	..	..	..	..	..	Ant.
XIX	..	o	c <sup>2</sup>	c <sup>1</sup>	o	..	..	..	..	..	..	..	Median.
XX	..	o	c <sup>2</sup>	c <sup>1</sup>	c <sup>2</sup>	o	..	..	..	..	..	..	Ant.

O denotes that no venomotor fibres run in the nerve designated, c that stimulation of the nerve gives a contraction of the vein. The exponents 1, 2, 3, indicate the strength of the contraction, 1 standing for the strongest. Langley's (*Journal of Physiology*, 1894, xvii, p. 296) classification of the different types of the lumbo-sacral plexus is followed.

The limit below which no venomotor fibres enter the sympathetic cannot be determined with certainty because it is masked by the fibres descending the sympathetic trunk from the upper white rami. Thus the constriction obtained by stimulation near what should be the lower limit cannot be used as evidence, for it may be the result of the stimulation of these descending fibres which have entered the sympathetic higher up.

From the III to the VI lumbar ganglion the venomotor fibres are found in the main trunk of the sympathetic; they have not yet begun to leave the sympathetic by the gray rami. The evidence for this



consists in the fact that the stimulation of any part of the main trunk of the sympathetic between the III and the VI lumbar ganglia was always followed by contraction, when the cat was in good condition, and section of the main trunk below the point of stimulation always prevented subsequent contraction. This evidence is conclusive, but it may be added that stimulation of the inferior mesenteric ganglia or any of the nerves connected with it invariably gave negative results.

Let us now inquire by what rami the venomotor fibres leave the sympathetic. The results of stimulating the gray rami communicantes of the spinal nerves forming the lumbo-sacral plexus are brought together in Table II. The rami were not stimulated directly, but the main trunk of the sympathetic was cut above and below the ganglion the ramus of which it was desired to investigate, and stimulated above the ganglion. In the case of the sacral rami, however, it was found inexpedient to cut the main trunk below the ganglia, so that the contractions recorded stand not only for these rami but also for any lower ones that may contain venomotor fibres. But on account of the general absence of these fibres in the II sacral and their occasional absence in the I sacral ramus there is no likelihood of their occurrence in any nerves below the II sacral. In stimulating the II sacral the general method was also deviated from in another respect. Instead of cutting and stimulating the sympathetic below the I sacral ganglion, which would have been difficult, the nerve was first stimulated above the I sacral ganglion and then its ramus severed, or easier still the whole spinal nerve severed, and stimulation repeated at the same place.

It will be seen from Table II that the venomotor fibres reach the sciatic by the rami to the VI and VII lumbar and the I and II sacral nerves. In the same animal, two, or more frequently three, rami contain these fibres, but in no case have they been found in all four rami. In every case the VII lumbar ramus contained venomotor fibres, while the VI lumbar and I sacral contained them in about eighty-five per cent of the cases. In one instance only was the II sacral found to give a contraction, but here, although the length of vein influenced was but one or two millimetres, the constriction was very distinct.

The most noticeable feature of the contractions obtained by stimulating the gray rami is their local nature. While the constriction upon stimulating the sciatic or sympathetic is several centimetres in

TABLE II.

Number of Experiment.	Rami to Lumbar and Sacral Spinal Nerves.						Character of Plexus.
	iv	v	vi	vii	i	ii	
XXII . .	o	o	..	..	..	..	Med.
XXXII . .	..	..	o	c <sup>a</sup>	c <sup>p</sup>	..	Post. a
XXXIII . .	..	..	o	c <sup>ap</sup>	o?	..	Ant.
XXXIV . .	..	o	c <sup>a</sup>	c <sup>p</sup>	o	..	Ant.
XL . .	..	..	..	..	c	o	Post. a
XLI . .	o	o	..	..	..	..	Med.
XLIII . .	..	..	c <sup>a</sup>	c <sup>ap</sup>	c?	..	Ant.
XLIV . .	..	..	c <sup>a</sup>	c <sup>ap</sup>	c	o	....
XLV . .	..	o?	c <sup>a</sup>	c <sup>ap</sup>	o?	..	Ant.
XLVI . .	..	o	c <sup>a</sup>	c <sup>ap</sup>	c <sup>ap</sup>	..	Ant. 14 thoracic vert.
XLVII left	..	..	c <sup>a</sup>	c <sup>a</sup>	o	..	Post. a
XLVII right	..	..	..	..	c <sup>p</sup>	..	....
XLVIII left	..	..	o?	c <sup>ap</sup>	c <sup>ap</sup>	c <sup>a</sup>	Ant.
XLVIII right	..	..	o	c <sup>a</sup>	c <sup>ap</sup>	c <sup>p</sup>	....
XLIX . .	..	..	c	c <sup>a</sup>	c <sup>p</sup>	o	Post. a
L . .	..	..	c?	c	c	o	Post. b

O denotes that the ramus was stimulated and no contraction obtained, although the stimulation of other nerves produced contraction. C means that the contraction was obtained by stimulating the ramus indicated. Exponents a, p, mean that the anterior or posterior veins only contracted; where no exponent is given, the condition of the cat was such that the localization observed was probably not significant.

length, the constriction caused by the stimulation of some of the rami is but a few millimetres long, and the region affected by one ramus is frequently different from that affected by another. The VII lumbar ramus controls a greater portion of the veins examined than any of the others, though occasionally the I sacral may equal or even exceed it in importance. The region controlled by the VI lumbar ramus is almost invariably quite small, and is confined to the anterior part of the leg. The transition from the contracting to the

inert region is often most abrupt, so that there is not the least difficulty in tracing the distribution of the fibres; but it may also be so gradual that it cannot be definitely located. When the contracting regions are well marked off from the inert ones it can be seen that sometimes the regions controlled by the VI and VII rami overlap, and that sometimes one stops almost exactly where the other begins.<sup>1</sup> But probably more frequent than either of these two arrangements is the one in which the VII ramus constricts the whole of the region that is affected, the anterior part of the same region being also controlled by the VI ramus. The relations between the VII lumbar and I sacral rami are not so definite, though occasionally similar phenomena are observed. In fact all the contractions of the posterior veins are usually less definite and clear-cut.

The constancy in the control of the anterior veins by upper rami is somewhat surprising in view of the variability in other respects. The only decided deviation from this control was in the II sacral ramus (Exp. XLVIII, left). Even in this case, however, the other side of the same animal possessed the normal arrangement.

The most variable quantity in the whole process is the size of all the regions that contract no matter what nerves are stimulated. From a good many cats no contraction at all can be obtained, and from this wholly negative result to the condition in which stretches of eight to ten centimetres contract strongly and uniformly there is every gradation in the size of the contracting region. Even in this variability, however, there is the constant feature that whenever there is any contraction at all it is almost sure to occur at about the middle of a superficial vein on the lateral side of the lower end of the crus, extending from the posterior edge of this member diagonally downwards and forwards to the upper extremity of the foot. When a greater part of the vein contracts it is usually this same region which contracts most strongly; and it is also to this place that fibres from both the VI and VII rami are distributed.

Another variable feature, which may depend somewhat upon the one just discussed, is the number and arrangement of the rami that produce a contraction. A rather close direct correlation between these and the anterior or posterior arrangement of the plexus would be expected, but Table II shows that there is no such correlation, so far as the small number of observations will allow us to judge.

<sup>1</sup> In several such cases I have subsequently stimulated the sciatic and found that it caused contraction of both these sharply differentiated regions.

The whole path of the venomotor fibres from their origin in the spinal cord to their termination in the veins of the hind limb is apparently made up of two neurons. The cell body of one of these neurons lies in the spinal gray matter; its axis-cylinder process, as I have shown, passes through the anterior root of one of the I, II, III, or IV lumbar nerves and the corresponding white ramus into the sympathetic chain, down which it runs for a certain distance, as described above. The cell body of the second or peripheral or sympathetic neuron lies in one of the sympathetic ganglia. The position of these ganglia was determined by Langley's nicotine method. After painting the III, IV, and V sympathetic ganglia, the stimulation of the pre-ganglionic fibres still causes constriction of the veins. The peripheral nerve cells are consequently not in these ganglia. Painting the VI and VII ganglia, however, renders the stimulation of pre-ganglionic fibres ineffective — the veins do not constrict. It is in one or both of these ganglia, then, that the peripheral venomotor neurons for the veins examined have their cells of origin, and it is here that the axis-cylinder process of the spinal venomotor neuron ends. This at least is true of all the cases I have examined, but it is possible that in Langley's more posterior types of the plexus some peripheral neuron cells may lie in the I and II sacral ganglia. This is suggested by the course of the post-ganglionic fibres.

The post-ganglionic fibres usually leave the sympathetic by the gray ramus immediately below the ganglion in which their cells of origin are situated. Thus when the sympathetic trunk is cut both above and below either the VI or the VII lumbar ganglion, the stimulation of the pre-ganglionic fibres between the section and the ganglion causes constriction. When, however, the ganglion is painted with nicotine the stimulation is usually, but not always, ineffective. This shows that the cells of the distal venomotor neurons are usually in the ganglion just above the ramus through which the fibres leave the sympathetic, but that occasionally they are located in a ganglion higher up. Since it has already been shown that stimulation of the I sacral gray ramus usually, and of the II exceptionally, causes constriction, and since, as has just been pointed out, the cells of origin of post-ganglionic fibres are usually situated in the ganglion immediately above the gray ramus in which they are contained, it follows that peripheral neuron cells may be situated in the I and II sacral ganglia, although I have not been able to demonstrate them with the nicotine method.

In general the arrangement of the venomotor nerves here described corresponds to that of the arterial vasomotor and sweat fibres of the hind limb.<sup>1</sup> The location of the ganglion cells and the course of the fibres through the gray rami is the same as that of the arterial vasomotor fibres, except that the stimulation of the II sacral ramus does not usually produce a contraction of the veins; but on the other hand the origin of the venomotor fibres from the spinal cord is more restricted. Bayliss and Bradford,<sup>2</sup> experimenting on the dog, found vasomotor fibres in the XI thoracic to the III lumbar spinal nerves, and Langley, who used cats, found them in the XII thoracic to the IV lumbar, whereas I have found them only in the I to IV lumbar nerves and have obtained the maximum effect from the III lumbar. It is evident, therefore, that the fibres to the superficial veins of the hind limb originate from the lower end of the region supplying all the vasomotor nerves for that member.

In conclusion I wish to express my thanks to Dr. W. T. Porter, at whose instance this work was undertaken and under whose direction it was carried on.

<sup>1</sup> Compare LANGLEY: *Journal of physiology*, 1891, xii, p. 347; *ibid.*, 1891, xii, p. 375; *ibid.*, 1894, xvii, p. 296.

<sup>2</sup> BAYLISS and BRADFORD: *Journal of physiology*, 1894, xvi, p. 10.

# AN ANALYSIS OF THE ACTION OF THE VAGUS NERVE ON THE HEART.

By L. J. J. MUSKENS.

[From the Laboratory of Physiology in the Harvard Medical School.]

	Page
I. Methods of investigation . . . . .	488
1. The stimulation of the vagus . . . . .	488
2. The method of recording contractions . . . . .	490
3. The preparation of the experimental animal . . . . .	492
II. The effect of vagus excitation on the interval between the contractions of the several parts of the heart . . . . .	493
1. The auriculo-ventricular interval . . . . .	493
2. The sino-auricular interval . . . . .	495
3. The interval between the contractions of the different parts of the sinus . . . . .	497
III. The influence of the vagus nerve on the force of the heart-beat . . . . .	497
1. On the force of the ventricle . . . . .	497
2. On the force of the auricle . . . . .	499
3. On the force of the sinus . . . . .	500
IV. The vagus influence on the frequency with which the sinus contracts . . . . .	500
V. The action of the vagus explained by its influence on the conduction of the cardiac excitation wave . . . . .	502
1. The conduction of the excitation process . . . . .	502
2. The force of contraction . . . . .	505
3. The rate of beat . . . . .	506

THE well-known experiments of Gaskell and Engelmann have convinced a great number of physiologists that the regular sequence with which the several parts of the heart contract is dependent on the periodic discharge by the sinus of an impulse to contraction, and the transmission of this impulse to the auricle and ventricle.<sup>1</sup> It has been demonstrated that the excitation process, measured by

<sup>1</sup> For the literature concerning the transmission of the cardiac excitation wave the reader is referred to BAYLISS and STARLING: *Internat. Monatshefte f. Anat. u. Physiol.*, 1892, ix, p. 256; also *Journal of physiology*, 1892, xiii, p. 407. BURDON-SANDERSON and PAGE: *Journal of physiology*, 1879-80, ii, p. 384. ENGELMANN: *Archiv f. d. ges. Physiol.*, 1875, xi, p. 465. *Ibid.*, 1878, xvii, p. 68; *Ibid.*, 1894, lvi, p. 149. *Ibid.*, 1896, lxii, p. 543. *Ibid.*, 1896, lxxv, p. 109. FANO: *Archiv ital. de biologie*, 1890, xiii, p. 387. GASKELL, *Journal of physiology*, 1883, iv, p. 43. MCWILLIAM: *Journal of physiology*, 1886, vi, p. 226. *Ibid.*, 1888, ix, p. 345. MARCHAND: *Archiv f. d. ges. Physiol.*, 1877, xv, p. 511. WALLER: *Philosophical transactions*, 1889, clxxx, p. 169. WALLER and REID: *Philosophical transactions*, 1887, clxxiii, p. 215.

the action current, passes as a wave over the auricle and ventricle, and that this excitation wave may be delayed in its course by various artificial hindrances or by the stimulation of the vagus nerve. It has been demonstrated further that the normal interval between the contraction of the sinus and auricle, and the auricle and ventricle, can be increased by the stimulation of the vagi or by the same artificial means that delay the excitation wave in its course within the auricle and ventricle. Upon these facts the assumption is based that the interval between the contraction of the several parts of the heart is due to the delay of the excitation wave by poorly conducting structures uniting these parts. The lengthening of the interval by vagus excitation is explained by a partial blocking of the excitation wave at the sino-auricular or auriculo-ventricular junction. When the block is sufficiently complete, the excitation wave is wholly arrested and the part of the heart between the block and the apex ceases to beat until the block is removed. Irregularities in rhythm and the periodic grouping of beats have also been explained by variations in the conducting power.

Such are the main outlines of the theory of the heart-beat developed by Gaskell, Engelmann, and others.

When the experimental basis of this theory is examined, it is found that while the propositions just enumerated cannot, in my judgment, be denied, they rest as yet on methods and observations that are incomplete in several important respects. Thus the influence of the vagus nerve on the passage of the cardiac excitation wave was studied by Gaskell chiefly in the tortoise. It is desirable that these phenomena be systematically investigated also in the frog, the classical experimental animal. Again, Gaskell did not maintain the normal nutrition of the heart; indeed, the heart was usually wholly removed from the body. To this abnormal nutrition is to be ascribed the fact that my own observations, both in the present work, begun in 1896 in the laboratory of Professor Engelmann in Utrecht, and in my former research on the reflexes obtained by stimulating the frog's ventricle,<sup>1</sup> differ from those of Gaskell in several important respects. For example, I find that the vagus lessens the force of the ventricular beat in the frog, as Gaskell states,<sup>2</sup> only when the frog is bled, or the normal state otherwise impaired. Finally, Gaskell has not apparently systematically recorded in a large number of animals simultaneous

<sup>1</sup> MUSKENS: *Archiv f. d. ges. Physiol.*, 1897, lxvi, p. 328.

<sup>2</sup> GASKELL: *Journal of physiology*, 1883, iv, p. 88.

curves of the movements of the sinus, auricle, and ventricle in such a way that the interval between the contractions of the sinus and auricle could be accurately measured. The lack of systematic records of the movements of the sinus obviously precludes the study of details which have an important bearing on the theory of the heart-beat. Engelmann, on the other hand, has not especially studied the action of the vagus in the light of this theory.

For these reasons it has seemed best to submit the action of the vagus on the heart, as well as certain indissolubly connected problems, to a fresh analysis, using for this purpose methods free from the objections which can be urged against much of the work of previous observers. The fruits of this inquiry will assist, I trust, in establishing still more firmly the views set forth above, and will show that the various actions of the vagus nerve upon the heart can all be explained by changes in the conducting power.

## I. METHODS OF INVESTIGATION.

1. **The stimulation of the vagus.**—The methods by which the vagus nerve has been stimulated in systematic researches on animals are alike in that they all require the preparation of the nerve by a dissection often long and difficult. Even in the hands of a skilful experimenter the operation can hardly be performed without some loss of blood, and this interference with the circulation, as will be presently demonstrated, affects the action of the vagus nerve upon the frog's heart to an extent hitherto unsuspected.<sup>1</sup> The usual method of stimulation therefore is of little value for the careful analysis of the action of the vagus nerve upon the heart of the animal in which this action can be most satisfactorily studied.

Another though less important defect is that the two vagi often differ considerably in their influence over the heart even in the same animal. This difficulty may be overcome by the excitation of both nerves simultaneously, but the preparation of both nerves involves a greater injury than the preparation of one, and consequently a greater impairment of the nutrition of the heart, upon the full preservation of which depends the normal action of the nerve.

It is plain, then, that for careful work the usual means of vagus stimulation in the frog must be abandoned, and a method found that does not require dissection and that can be employed for both nerves at the same time. Such a method will be now described.

<sup>1</sup> Compare ENGELMANN: *Archiv f. d. ges. Physiol.*, 1894, lvi, p. 166.



Soon after leaving the cranial cavity the vagus nerve in the frog passes across the levator anguli scapulæ superioris muscle and touches the cartilaginous capsule which contains the middle ear. The nerves may here be approached from the inside of the mouth with electrodes adapted to the local conditions. If such electrodes are pressed gently against the posterior margins of the Eustachian tubes, a weak current will produce in an irritable frog a strongly marked vagus action upon the heart. Not infrequently the stimulation is still more effective when the electrode is placed outside the cartilage ring which supports the Eustachian tube. The electrodes employed (Fig. 1) were made of copper wire insulated by a block of hard rubber and by rubber tubes. The terminals were of lead. The lead terminal pressed with a certain slight force against the mucous membrane, thus making a better contact and assisting to immobilize the frog. For the smaller species of frog (*R. temporaria*, *R. esculenta*, *R. palustris*, etc.) the electrode should be one or two millimetres in diameter; for *R. catesbeiana* three or four millimetres.

It may be objected that the part of the medulla oblongata lying between the two electrodes is stimulated by this method, and not the vagus nerve itself. This objection is answered by the following experiment. The electrodes were applied in the manner above described and the minimal stimulation by which arrest of the heart could be produced was determined. The whole central nervous system was then thoroughly destroyed, care being taken not to displace the electrodes. As is always observed after the destruction of the vagus nuclei, the heart was arrested from two to four minutes. After the return of the heart-beat arrest could still be caused by vagus stimulation with the previous strength of current. Occasionally the arrest was more pronounced after the brain and cord were destroyed than before. This experiment was done in curarized and non-curarized *Rana catesbeiana* and *R. palustris*.

It may further be objected that the arrest is due to the escape of current to the heart itself. This objection is deprived of its force by

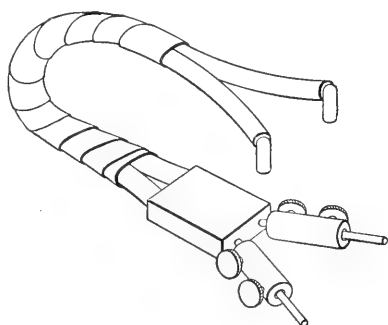


FIGURE 1. Electrodes for the stimulation of both vagi near the middle ear. About one half the actual size.

the observation that the effect of vagus excitation was not diminished by increasing the distance between the point of stimulation and the heart. On placing glass tubes 18 to 20 millimetres in diameter in the mouth and œsophagus of several *R. catesbeiana* the linear distance from the heart-root to the stimulating points exceeded twenty millimetres. The effects of stimulating such a preparation are usually more marked than when the œsophagus is not distended; the nerves are apparently rendered more irritable by being stretched over the glass tube. Additional evidence against the escape of current was secured by laying the nerve of an irritable nerve-muscle preparation upon the heart. Had the very weak stimulating current employed reached the heart, the gastrocnemius muscle must have contracted, yet no contraction occurred. Finally, in two *R. catesbeiana* very effective vagus excitations were produced when the secondary coil of the inductorium was at 80 and 100 millimetres from the primary. Then both vagi were cut. On repeating the stimulation no vagus effects could be observed even with induction currents of maximal force.

Still another criticism may be made. It may be said that the vagus effects observed were not the result of a direct but of a reflex excitation of the vagi, similar to the reflex excitation obtained by Goltz on striking the intestines. A sufficient answer to this has already been given in the observation that the vagus effect could be secured after the destruction of the cord and brain. It may be added that the latent period in direct stimulation of the nerve is markedly shorter than the reflex time, as determined for example by Engelmann and myself for the reflex from the stomach upon the heart (two to three seconds).

The last objection which occurs to me is that the stimulation by my method affects not merely the vagi but adjacent nerves as well, for example, the sympathetic. This is indeed the case, but it should be remembered that sympathetic fibres join the vagus trunk immediately after it pierces the skull. This objection can therefore be made against any method of vagus stimulation, except the intracranial one, which cannot be employed for the studies we are now making because the formidable operation which it requires disturbs the normal influence of the vagi upon the heart.

2. **The method of recording contractions.** — The movements of the cold-blooded heart may be recorded best by the so-called suspension method of Gaskell<sup>1</sup> modified by Engelmann. Engelmann's modi-

<sup>1</sup> GASKELL: *Journal of physiology*, 1883, iv, p. 43.

fication has important advantages: it does not interfere with the circulation of the blood; and it has been thoroughly studied with regard to its details. In the suspension method as employed by Engelmann the point of the ventricle *in situ* is pierced by a pin bent at an angle, and the pin is connected to the short arm of a light counterpoised lever. However rough it may seem superficially, the harmlessness and effectiveness of this suspension have been fully proved.<sup>1</sup>

For the present inquiry into the influence of the vagus on the contraction interval it was evidently necessary to record as precisely as possible the time elapsing between the beginning of the auricular and the beginning of the ventricular contraction. The suspension of the ventricle alone has the disadvantage that the deepest point in the curve does not coincide exactly with the beginning of the contraction of the auricle,<sup>2</sup> as can be seen in Engelmann's Fig. 5. In a great number of my experiments, therefore, the ventricle and auricle were suspended separately each to its own lever. By this procedure the auricular lever may be made to write the contractions of the sinus as well as the auricle. In the American bull-frog, which not infrequently attains the length of fifteen inches, the conditions for the record of the contractions of the sinus, auricle, and ventricle are very favorable indeed. After some experience contraction curves ten millimetres and more in height can be obtained from the sinus or one of the large veins. An extremely light lever with a very fine writing point is needed. Very often the movements of that part of the auricle which is closely connected with the sinus superimposes a catacrotic elevation on the sinus curve. This is, however, not an imperfection of method; on the contrary, it permits occasionally a more exact measurement of the interval between the beginning of the contraction of that part of the sinus which is suspended and the beginning of the contraction of the corresponding region of the auricle.

The greatest care should be taken not to injure the delicate muscular walls of the veins. The suspension clamp, made of German silver wire in the shape of a *serre-fine*, must not be allowed to include

<sup>1</sup> ENGELMANN: *Archiv f. d. ges. Physiol.*, 1892, lii, p. 357.

<sup>2</sup> This imperfection can be remedied to a great extent by making the heart contract more slowly. A test tube filled with ice and placed in the œsophagus will accomplish this by cooling the sinus. An additional advantage is thus secured, for the whole heart is raised upwards and the suspension of its various portions greatly facilitated.

a slip of the pericardial membrane. In all cases the pericardium is to be removed as far as possible before suspending.

3. **The preparation of the experimental animal.** — The immobilization requires great care. Destruction of the brain cannot be thought of; in the first place this operation cannot be done without an abundant extravasation in the brain cavity and the spinal canal, which, as will be seen, disturbs the normal action of the vagus upon the heart; in the second place the rough operation gives rise to countless efferent impulses, which cannot fail to exert an important influence upon so sensitive an organ as the heart. Ordinary doses of curare are also excluded. Curare administered in an amount sufficient to paralyze the voluntary muscles diminishes the reflex irritability and also the effectiveness of the vagus stimulation upon the heart. The most satisfactory manner of preparing the frog is to give an almost homœopathic dose of curare twelve to twenty hours before the experiment. The dose (which is to be determined for every one per cent curare solution used) is about .01 cubic millimetre. As a matter of course the larger animals need more of the poison than do the smaller ones. It can be given by a syringe subcutaneously, or be injected by a pipette into the dorsal lymph-sack. The drug administered in this manner has simply a restraining effect upon the voluntary movements. Within twenty-four hours the animal is generally perfectly restored. If the curare has totally paralyzed the animal in one hour, the chance for good results is already decreased.

The irritability of the experimental animals is a very variable factor. Especially is this true of the frog. In some seasons (spring) it may happen that the excitation of the vagi has a pronounced effect on the heart in almost every frog, while at other times only one animal in ten or twenty may possess sufficient irritability. It was sometimes possible to increase the irritability of the frogs by leaving them over night with exposed intestines in the moist chamber. The best test of a sufficient irritability is the occurrence of spontaneous arrest of the heart. This is often seen in fresh irritable frogs. Thus in slightly curarized animals movements of the limbs having the character of a movement of escape will take place, without any discoverable reason; a latent period follows and then arrest of the heart. Such arrest is often seen to recur in regular intervals of two or three minutes.

This spontaneous — I dare say physiological — arrest gave me an indication of what character and what duration artificially produced

arrest ought to be. The best arrest I hold to be that obtained with currents of minimal intensity and duration.

The vagi, or their terminations in the heart, are very soon fatigued. A rest of at least two minutes between successive stimulations is necessary.

The heart is to be kept moist. Very useful for this is a physiological gelatine solution (5 grams of gelatine dissolved with the aid of heat in 300 grams of normal saline solution).

## II. THE EFFECT OF VAGUS EXCITATION ON THE INTERVAL BETWEEN THE CONTRACTION OF THE SEVERAL PARTS OF THE HEART.

I. **The auriculo-ventricular interval.** — The interval between the contractions of the auricle and ventricle is usually prolonged when the vagi are stimulated. Sometimes, however, especially with very weak currents, the duration of the interval is not affected, although the force of the auricular systole is diminished. This is in fact a most

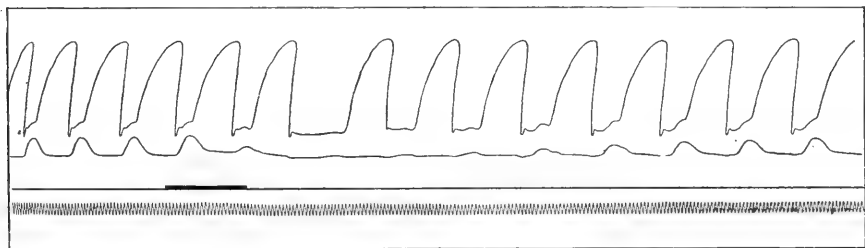


FIGURE 2. Four-fifths the original size. The uppermost curve records the movements of the ventricle (R. temporaria); the second curve, the movements of the auricle. The elevation in the third curve marks a reflex stimulation from the stomach (tetanization). The fourth curve was written by a tuning fork swinging 10 times a second.

common effect of weak vagus stimulation. In Fig. 2 the intervals are 43, 43, 42, 43, 61, 63, 69, 73, 66, 52, and 46 hundredths second, reading from left to right. As a rule the increase reaches its maximum rapidly and then slowly decreases. A second maximal value is often observed as an after effect.

Figure 3 is an example of the effect of a stronger vagus excitation. The height of contraction is diminished in the first auricular systole after the stimulation, but no measurable increase in the auriculo-ventricular interval is observed until the next cardiac cycle. Then the interval, which had been 39, 40, 40, 40, increases to 63 hundredths

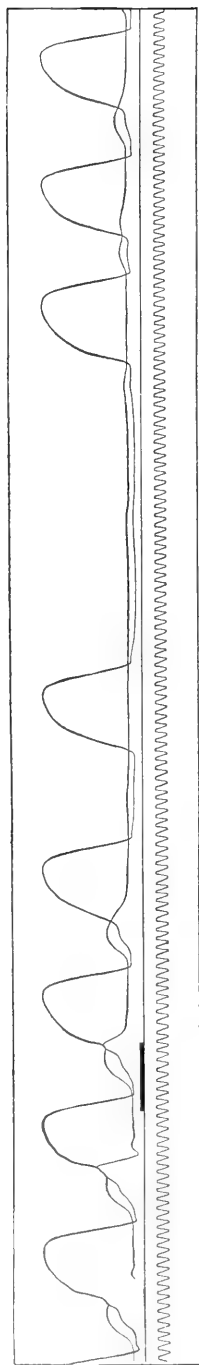


FIGURE 3. Two-thirds the original size. The same as Fig. 2, except that the stimulation was stronger.

second. The ventricle then loses one beat, while only a slight elevation in the auricular curve gives evidence of the systole of the auricle. In the next cycle also the ventricle does not beat, but the vagus influence is already waning, for the force of auricular systole is observed to increase. In the next cycle the ventricle itself contracts again, the auriculo-ventricular interval is still prolonged, being 0.51 sec. Gradually the normal interval and the normal force of auricular systole is restored.

Under still more powerful vagus excitation the auricular systoles become too small to be recorded. The first cycle after the so-called arrest is in such cases often incomplete. The ventricle beats, but the auricular contraction remains invisible, appearing first in the next cycle; or the auricular contractions become visible again, while the ventricular contractions do not yet appear. The effect of powerful vagus excitation on the auriculo-ventricular interval is shown in the accompanying table. This table will serve also to illustrate the method of collecting the data on which the conclusions in this paper rest. A very large number of such measurements were made. Space does not permit their presentation in full; and indeed their full presentation is unnecessary, for they only confirm the conclusion that any one must draw from an inspection of the curves.

The division of the vagus action into the three degrees described above is one of convenience merely. There is no essential difference between them. For the first and second degrees the curves demonstrate clearly that the influence of the vagus on the length of the auriculo-ventricular interval is of the greatest importance. In the third degree the ventricular beat is lost and the auricular

contractions are too small to be recorded; hence we cannot measure the contraction interval; it is, however, certainly prolonged. In the theoretical part of this paper, I shall point out how probable is the view that the apparent arrest of the ventricle is not really an arrest but a failure to be excited to contract, in consequence of the blocking of the excitation wave on its way from the sinus to the ventricle by the action of the vagus.

TABLE I.

Number of Experiment.	Date.	Animal.	Auriculo-ventricular interval in 0.01 sec. <sup>1</sup>											
			Before stimulation.		During and after stimulation.									
1	Jan. 18, 1896	R. temporaria	19	19	13	..	..	..	33	24	22	20	..	..
2	Feb. 30, 1896	R. temporaria	41	41	44	..	..	..	?	47	43	42	40	..
3	Dec. 2, 1897	R. catesbeiana	40	40	38	..	..	..	..	..	56	54	52	48
4	Dec. 7, 1897	R. catesbeiana	50	50	46	50	..	..	..	..	54	52	76	64

<sup>1</sup> It should be remarked that the points by which the duration of the contraction interval are measured cannot be located with an accuracy of 0.01 sec. It is not possible to determine so closely as this the exact moment of the beginning of the systolic rise in the curve. The reckoning has been expressed in 0.01 sec. merely to give accuracy to the tenths.

2. **The sino-auricular interval.** — In a second series of experiments the ventricle and auricle were again separately suspended, but the auricular suspension hook was so placed that the contractions of the sinus as well as the auricle appeared in the curve, thus affording the means for an analysis of the interval between the contractions of the sinus and the auricle as well as that between the contractions of the auricle and ventricle. Fig. 4 is an example. In this figure the points at which the contractions of the sinus, auricle, and ventricle begin are indicated by the first, second, and third vertical lines, respectively. The fourth and fifth lines mark the beginning of sinus and ventricle contraction in another period. The sinus continues to beat notwithstanding the vagus excitation, but is much reduced in force, being in the 5th cycle scarcely visible. The force of the auricle diminishes sooner—in the 4th cycle; and in the 5th, 6th, 7th, 8th, and perhaps the 9th, no auricular systole can be seen. In

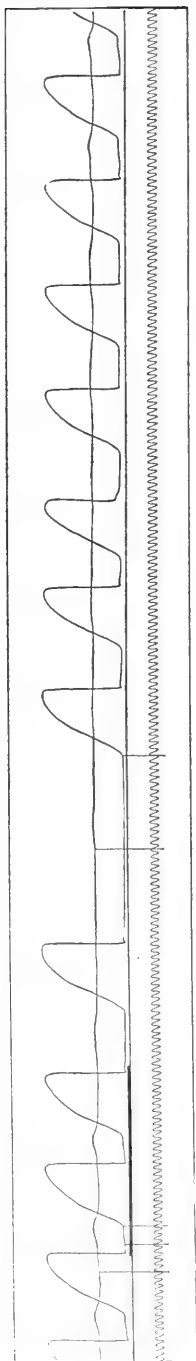


FIGURE 4. Three-fifths the original size. The uppermost curve records the contractions of the ventricle; the next, those of the sinus and auricle; the rise in the third curve marks the reflex stimulation of the vagi (from the stomach); the lowermost curve gives the time in tenths of seconds. The vertical lines indicate the points at which the contractions of the sinus, auricle, and ventricle begin.

the 10th cycle the contraction of the auricle is again visible in the curve and thereafter the force of contraction is slowly regained. The sino-auricular interval cannot be measured here directly, because the auricular contractions are not recorded, but the increase in the interval may be demonstrated by the increase in the interval between the contractions of the sinus and that of the ventricle. If it is urged that this is not a rigid proof, because the lengthening of the sino-ventricular interval may be due simply to the lengthening of the auriculo-ventricular interval, the measurements in Table II, 3 and 4, will be a sufficient answer. These cases are from experiments, in which the auricular contraction, though reduced in height, was still sufficiently visible to make the identification of its beginning-point possible. The interval between sinus systole and auricular systole is considerably increased in case 4. The vagus therefore possesses the power of lengthening the sino-auricular as well as the auriculo-ventricular contraction interval.

The lengthening of these contraction intervals may be regarded as the usual result of vagus excitation. It is, however, not the invariable result. The contraction interval is sometimes decreased. It may even be decreased between sinus and auricle, and at the same time increased between auricle and ventricle, as shown in Fig. 5, or the reversed effect may be observed. The uppermost curve in this figure shows the contractions of the auricle and ventricle, the lowermost curve those of the sinus. The sino-auricular intervals from left to right are 72, 72, 70, 48, 70, and 68 hundredths second, while the auriculo-



ventricular intervals are 42, 44, 42, 50, 46, and 48 hundredths seconds (see Table II, 1 and 2).

3. The interval between the contractions of the different parts of the sinus. — Not only may the intervals between the contraction of the main anatomical divisions of the heart, namely, the sinus, auricle, and ventricle, be altered by vagus influence, but the interval between the contractions of different parts of the sinus as well. Evidence will be presented later (Fig. 9) to show that different parts of the sinus may be dissociated by vagus excitation, so that they beat at different times and not practically together as they ordinarily do. The important bearing of this dissociation will be discussed in section V.

### III. THE INFLUENCE OF THE VAGUS NERVE ON THE FORCE OF THE HEART-BEAT.

1. On the force of the ventricle.—The vagus is said by Coats,<sup>1</sup> Heidenhain,<sup>2</sup> Gaskell,<sup>3</sup> Hofmeister,<sup>4</sup> and others, to

The sino-aortic and aortic-ventricular contraction intervals of successive cycles (in 0.01 seconds).																						
Number of Experiment.	Date.																					
		1		2		3		4		5		6		7		8		9		10		
		$S-A$	$A-T'$	$S-A$	$A-T'$	$S-A$	$A-T'$	$S-A$	$A-T'$	$S-A$	$A-T'$	$S-A$	$A-T'$	$S-A$	$A-T'$	$S-A$	$A-T'$	$S-A$	$A-T'$	$S-A$	$A-T'$	
1	Nov. 26, 1897	36	18	32	18	33	17	..	..	20	20	21	20	24	18	25	19	30	17	32	18	
2	Nov. 26, 1897	44	11	46	11	..	11	..	..	30	15	31	19	31	18	35	17	38	17	38	17	
3	Feb. 6, 1896	50	31	52	32	50	40	101	..	56	50	55	49	50	39	50	30	..	..	..	..	
4	Feb. 5, 1896	48	41	50	41	..	..	..	..	81	43	73	42	66	44	63	46	56	43	..	..	

<sup>1</sup> COATS: Berichte d. k. Sächs. Gesellsch. d. Wissensch., math.-phys. Cl., 1869, p. 370.

<sup>2</sup> HEIDENHAIN : Archiv f. d. ges. Physiol., 1882, xxvii, p. 395.

<sup>3</sup> GASKELL : *Journal of physiology*, 1883, iv, p. 88.

<sup>4</sup> HOFMEISTER: Archiv f. d. ges. Physiol., 1889, xliv, p. 420.

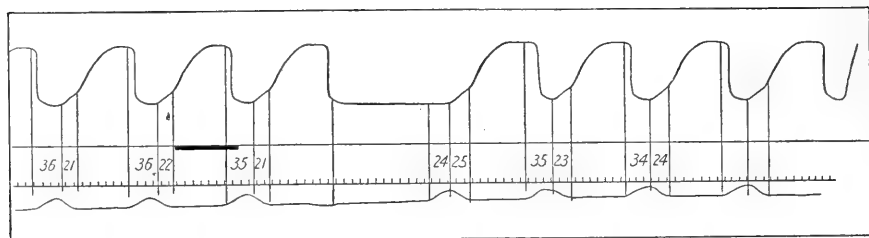


FIGURE 5. Four-sevenths the original size. The uppermost curve records the contractions of the auricle and ventricle; the fall in the next curve marks the duration of vagus excitation; the third curve gives the time in fifths of seconds; and the lowermost curve records the contractions of the sinus (vena cava superior sinistra).

lessen the force of ventricular contraction in the frog. All these investigators employed the excised heart or prepared the vagi without especial regard to the loss of blood. My work with both Dutch and American frogs has shown that the loss even of a little blood alters the action of the vagus on the force of ventricular contraction. Under normal conditions of nutrition I find among thousands of records from four different species of *Rana*, not one in which vagus excitation diminishes the force in the manner described by these authors.<sup>1</sup> The ventricle, it is true, may be arrested altogether, but this, according to the hypothesis to be presently discussed, is not the result of a reduction in force to the point at which a contraction is no longer possible, but the failure of the excitation wave to reach the contractile substance. Sometimes the height of one or more ventricular systoles after the so-called arrest is found to be irregular—in case the ventricle has missed several beats. The extreme distention of the ventricle with venous blood—mechanically preventing the full effect of the contraction,<sup>2</sup> the slight weakening of the muscle by interference with its nutrition, and the variation in the rate of beat are probable explanations of these irregularities. The simple experiment of bleeding a frog during a continuous series of vagus stimulations has never failed to show that so soon as the normal nutrition is altered

<sup>1</sup> GASKELL, *loc. cit.*, p. 89, found that the vagus does not influence the force of ventricular contraction in the tortoise; MCWILLIAM, *Journal of physiology*, 1885, vi, p. 223, reached the same result for the eel's ventricle. BAYLISS and STARLING, *Journal of physiology*, 1892, xiii, p. 411, and NUEL, *Archiv f. d. ges. Physiol.*, 1874, ix, p. 186, deny the power of the vagus over the force of contraction of the ventricle. ROY and ADAMI, *Philosophical transactions*, 1892, clxxxiii, p. 224, doubt its power.

<sup>2</sup> ENGELMANN, *Archiv f. d. ges. Physiol.*, 1894, lvi, p. 182, points out that the intra-cardiac pressure influences the auriculo-ventricular contraction interval.

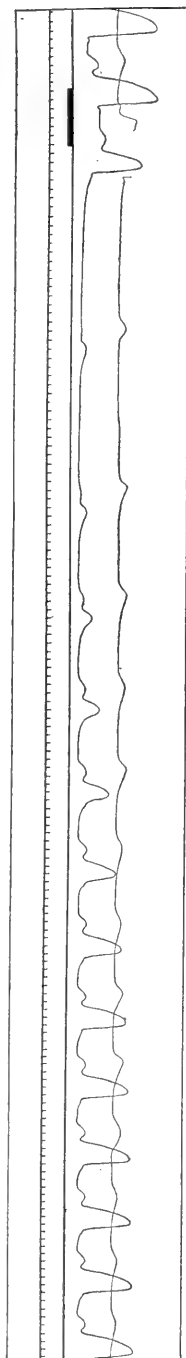
even slightly a decrease of the force of ventricular contraction appears. Fig. 6, taken from a frog that had been bled, shows this influence of the vagus stimulation on the force of the ventricle. The last systole before the arrest is already decreased in height.

Sometimes after the arrest the frequency is increased and the force diminished (see Fig. 11, page 507). The latter effect is, I believe, purely secondary. The ventricle beats so quickly that the force of the individual beat is lessened. The increase in frequency is in consequence of interference; the accelerators in the vagus trunk, contrary to the usual rule, probably overcome the inhibitory fibres.

Occasionally though rarely the first contractions after arrest are increased instead of diminished in force. In my experience the increase in force has always been of an irregular type, and associated with a slowing in the rate. The explanation of the increased force is probably the fact established by Gaskell for the tortoise and by McWilliam for the warm-blooded heart, namely, that normally the frequency of beat is too great to permit the ventricle to contract with maximal force. The slow rate under vagus excitation may favor thus the development of the maximal contraction.

2. *On the force of the auricle.*—The action of the vagi upon the force of the auricular contraction has been demonstrated too often to require discussion here. The influence seems to be of the same general character before and after bleeding, although in the latter case it is more marked. As in the ventricle, so

FIGURE 6. Five-pinths the original size. The uppermost curve records the movements of the sinus (vena cava superior dextra); the second curve the movements of the auricle and ventricle; the depression in the third marks the duration of vagus excitation; the lowermost curve marks the time in fifths of seconds.



here the maximum is quickly reached and the previous force regained slowly.

3. **On the force of the sinus.** — The vagi diminish the force of the sinus contraction also; the effect is more quickly produced than in the auricle and ventricle. At times a simple reduction in the height of the curve is observed; at others the systolic rise is no longer single — not one but several elevations are seen at each systole (see Fig. 7). This division of the systolic curve into several parts indi-

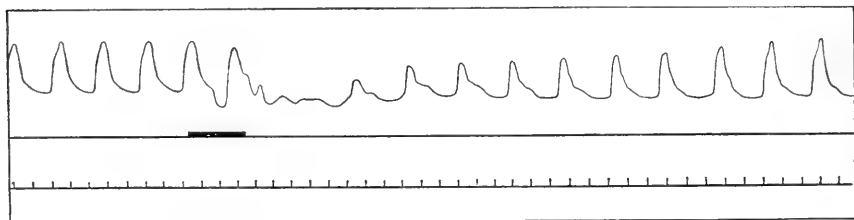


FIGURE 7. Three-fourths the original size. The uppermost curve records the movements of the sinus (vena cava inferior) of *Pseudemys elegans*; the rise in the second curve marks the duration of vagus excitation; the lowest curve marks the time in fifths of seconds.

cates that the different portions of the sinus no longer contract in their normal sequence, but are dissociated by the vagus. The theoretical importance of this observation will be insisted on hereafter.

#### IV. THE VAGUS INFLUENCE ON THE FREQUENCY WITH WHICH THE SINUS CONTRACTS.

It will be observed that the title of this section contains no reference to the frequency of the contraction of the auricle and the ventricle. The omission is a logical consequence of the hypothesis which my experiments have forced upon me more and more strongly, namely, that the normal heart-beat is dependent primarily upon impulses discharged by the sinus. This being accepted — and the hypothesis is accepted by a strong school of physiologists — it follows that changes in the frequency of contraction of the auricle and ventricle in consequence of vagus excitation are secondary effects and not the result of the action of the vagi on the production of impulses by the auricle and ventricle. There are two ways in which these secondary effects may be brought about: the vagi may hinder the transmission of the excitation wave and thus delay its arrival in the contractile substance of the auricle or ventricle; or, secondly, the stimulated

vagi may induce intrinsic changes in the auricle and ventricle affecting the character and time-relations of the discharge occasioned by the excitation wave on its arrival in those parts. This being the point of view, we may reserve the consideration of changes in the rate of auricular and ventricular contraction for the discussion of the theoretical bearings of my work. For the present we need only inquire whether any changes in the rate of beat of the sinus are seen when the vagi are stimulated.

The fact is that the rate is often changed. The excitation of the vagus produces sometimes an increase in the frequency of sinus contraction, but usually a decrease.

The occasional increase in the frequency of sinus contraction may be accompanied by an increase in the frequency of the auricle and ventricle. Once I saw after a powerful stimulation of the vagus near its entrance into the sinus a very marked quickening of the heart-beat; this lasted until I stimulated a second time in the same manner and with the same current; then a very marked slowing followed.

Both Dutch and American frogs have furnished me curves in which acceleration and retardation of the whole heart-beat succeed each other suddenly (Fig. 8). This well-known phenomenon is probably

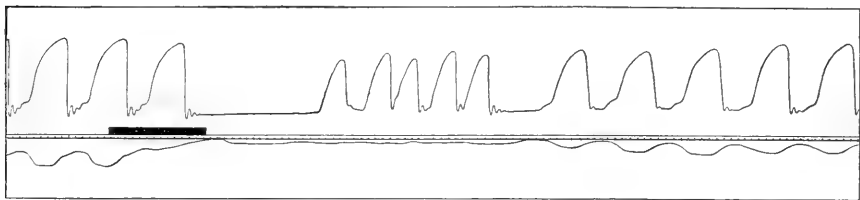


FIGURE 8. One-half the original size. The uppermost curve records the movements of the auricle and ventricle; the rise in the second curve marks the duration of vagus stimulation; the third curve is the time in fifths of seconds; the lowest curve records the movements of the sinus (*vena cava superior sinistra*).

the result of the interference of retarding and accelerating heart nerves. A satisfactory explanation, in my judgment, can be found in the variation of the conducting power. As soon as the conducting power has increased to a certain point the excitation process, previously blocked, flows over the whole heart and an increase in the number of visible contractions results.

The alternating of arrest with augmented rhythm is apparent rather than real, and is to be explained by changes in the conducting

power in consequence of which the contraction wave is sometimes carried to the ventricle and at other times does not extend beyond its origin in the sinus.

#### V. THE ACTION OF THE VAGUS EXPLAINED BY ITS INFLUENCE ON THE CONDUCTION OF THE CARDIAC EXCITATION WAVE.

The basis of the hypothesis about to be discussed is the widely accepted theory that the excitation process which occasions the heart-beat arises automatically in the sinus and from its point or points of origin overflows first the sinus, next the auricle, and lastly the ventricle. In agreement with this theory, the arrest of any part of the heart by the vagus may be explained, first, by the blocking of the excitation wave between its point of origin and the part arrested, so that the latter receives no impulse to contraction; secondly, by a reduction of irritability<sup>1</sup> or other intrinsic change in the arrested part so great that the excitation wave upon its arrival there is not able to occasion any measurable alteration in form. I can offer no rigid demonstration of the truth or error of these hypotheses, but a careful study of the facts leads me to the conclusion that all the various phenomena can be explained by changes in the conduction process so readily and with such close adherence to established facts and scarcely less established points of view as to make this explanation much more probable than the alternative one of intrinsic changes in the several parts of the heart.

I. **The conduction of the excitation process.**—Let us begin our analysis by attempting to explain changes in the duration of the contraction interval by changes in the conduction of the excitation process. In Fig. 9 the uppermost curve records the movements of the auricle and ventricle, the lowermost curve the contractions of the sinus. The time is marked by the chronograph of Jaquet recording fifths of seconds. The current derived from two Daniell cells was conveyed through the primary circuit of an induction apparatus and through an electromagnet which recorded the time of stimulation. The distance between the coils of the induction apparatus was 120 millimetres at the beginning of the stimulation but was slowly reduced to 75 millimetres. On examining the curve it will be seen that the excitation is followed by one normal beat, after which the contractions of the auricle and ventricle disappear from the curve. The elevation observed in the sinus curve is due to the movements of voluntary

<sup>1</sup> Compare McWILLIAM: *Journal of physiology*, 1888, ix, pp. 352, *et seq.*

muscles, very often seen in reply to stimulation. Upon close examination, it will be noticed that after the standstill the whole sinus does not contract at the same moment. A small, sharply defined elevation precedes the first large contraction of the sinus by 1.68 secs.; shortly afterward, the contraction wave pursues its regular way over the auricle and ventricle. In the next cardiac cycle, a similar small elevation precedes the main contraction of the sinus by 1.34 secs.,—a slight reduction. In the following cycles, the interval between the two parts of the sinus movement falls to 1.14, 0.82, 0.40, 0.28, 0.18, 0.12 secs., and finally the interval disappears and the whole sinus contracts apparently simultaneously.

Evidently the excitation of the vagus in this case dissociated one part of the sinus from another.<sup>1</sup> The normal rapid conduction of the excitation wave in the sinus is here diminished by vagus excitation, just as it has been shown by Engelmann, Gaskell, and others to be diminished in the auricle and ventricle. In consequence of the slower conduction the interval between the part of the sinus first reached by the excitation wave and that reached later is such that the two contractions are no longer fused into an apparently single contraction but are seen separately in the curve. As the vagus influence wanes, the conducting power gradually returns to normal, and the interval between the contraction of the several parts of the sinus becomes less

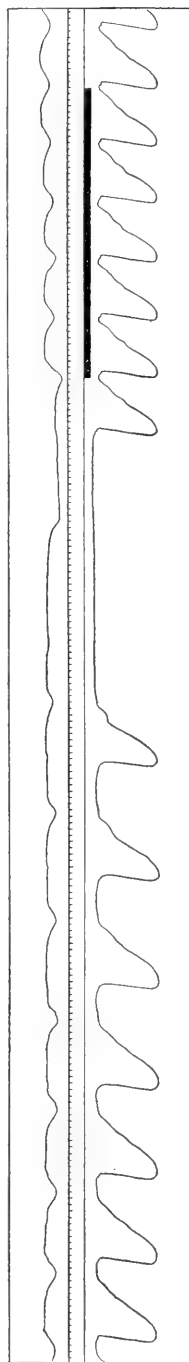


FIGURE 9. Five-ninths the original size. The uppermost curve records the contractions of the auricle and ventricle; the rise in the second curve marks the duration of vagus stimulation; the third gives the time in fifths of seconds; the fourth the contractions of the sinus (vena cava superior sinistra). R. catesbeiana.

<sup>1</sup> MCWILLIAM: *ibid.*, 1886, vi, p. 220, observed a similar dissociation in the auricle and sinus of the eel's heart.

and less until fusion again takes place. Sometimes, after the so-called arrest, the sinus is seen to contract in three divisions, and indeed it is entirely probable that the dissociation of the sinus contraction under nerve influence may proceed much further than can be demonstrated by our present methods of research.

Experiments on the sinus in turtles (*Pseudemys rugosa* and *elegans*) confirmed the results secured with frogs. In the turtle I succeeded in suspending two different parts of the sinus, *i. e.* two parts of the vena cava inferior, at the same time. Curves were thus obtained which showed that the sinus and the large veins are not one contractile body but a system of contractile units. The contraction wave in most cases was seen to start from the right vena cava. The separation into contractile units may be recognized not only when the heart is exhausted after bleeding (as shown by Engelmann in the frog) and in consequence of vagus stimulation as above described, but also under apparently normal conditions. It seems most probable that the time elapsing between the contractions of the different parts is dependent on the conducting power between these parts.

The reader may be reminded here that antiperistaltic contractions of the sinus and large veins may be observed quite frequently in exposed hearts which are beating normally. I have seen these in the turtle; Engelmann<sup>1</sup> observed antiperistalsis in the frog and Bottazzi<sup>2</sup> in the heart of the chick. The possibility of the occurrence of this phenomenon should be taken into account in the discussion of the cases in which the vagus nerve seems to have a direct influence on the production of automatic stimuli.

If the vagus, as has just been shown, can thus separate the contractions of various portions of the sinus so that a contraction curve that normally appears single becomes divided into several parts, and if, as has been shown in section second, the vagus can also increase the normal interval which separates the contraction of the sinus from that of the auricle, and the contraction of the auricle from that of the ventricle, it is certainly reasonable to suppose that a strong excitation of the nerve may interpose such a resistance to the passage of the excitation wave as to block it entirely. The parts of the heart which cease to receive the excitation, or receive less than the amount sufficient to cause a measurable change in form, will then cease to record

<sup>1</sup> ENGELMANN: Archiv f. d. ges. Physiol., 1895, lxi, p. 275.

<sup>2</sup> BOTTAZZI, P.: Sullo sviluppo embrionale della funzione motoria, Firenze, 1897, p. 78.



their contractions, as, for example, in Fig. 9 the contractions of the auricle and ventricle disappear from the curve for a time, while the sinus goes on beating.

Decreased conducting power within the limits of the sinus will further explain the simple prolongation of one or two cycles often seen as an effect of weak vagus excitation (Fig. 8).

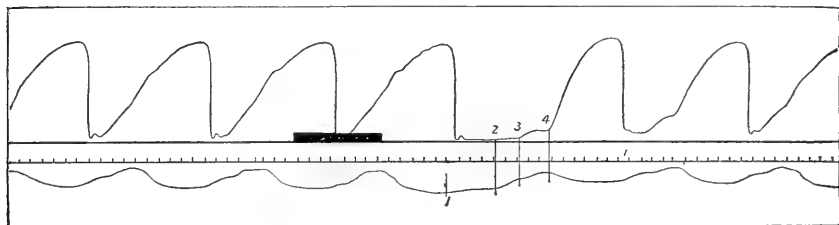


FIGURE 10. The uppermost curve records the contractions of the auricle and ventricle; the rise in the second curve marks the duration of vagus excitation; the third marks the time in fifths of seconds; and the lowermost curve the contractions of the sinus (vena cava superior sinistra). The vertical lines 1, 2, 3, 4 mark the beginning of contraction of the smaller part of the sinus, the greater part of the sinus, the auricle, and the ventricle, respectively.

It also explains numerous instances occurring in *R. catesbeiana* as well as *R. temporaria* in which the duration of the cardiac cycle after stimulation is nearly or exactly twice that observed before stimulation (Fig. 5).

2. **The force of contraction.**—The observation that the sinus is really a composite of contractile units, together with the observation that the vagus influences these functional units in different degrees, thus dissociating them from their normal relation, suggests the possibility that the vagus nerve may regulate the force of the cardiac contraction simply by changing the conducting power.

The diminishing force of contraction under vagus inhibition may mean that fewer and fewer fibres take part in the contraction, because the decreasing power of conduction prevents more and more fibres from being reached by the excitation wave.<sup>1</sup> The return of force after vagus inhibition may mean that the improving conducting power enables the excitation to reach a constantly increasing number of fibres. This view is strengthened by the similarity in the action of the vagus on the force of contraction and on the length of the contraction interval; in both the effect rises quickly to a maximum and

<sup>1</sup> Compare ENGELMANN: *Archiv f. d. ges. Physiol.*, 1896, lxii, p. 555.

then slowly disappears. It is further strengthened by the fact that in frogs which have been bled the force of ventricular contraction diminishes nearly *pari passu* with the lessening in conducting power between sinus and auricle, and auricle and ventricle (measured by the increase in the contraction interval).

The diminishing force of contraction may also be explained by the failure of the several parts of the sinus to beat in unison (Fig. 7).

It seems not improbable that the "staircase phenomenon" may be explained by a gradual increase in conductivity enabling each successive excitation wave to reach farther and farther into the ventricle.<sup>1</sup> In this connection it may be remembered that I described in Pflüger's Archiv<sup>2</sup> a peculiar direct effect of tetanizing currents sent through the ventricle, which is to be seen whenever the nutrition of the heart (and also the conducting power) is diminished. I saw the force of the ventricle quickly decrease, and, after the tetanizing, slowly increase again, in other words, a staircase phenomenon. This also may be regarded as due to changes in the conductivity.

3. **The rate of beat.**—The facts already stated warrant the assumption that variations in the duration and the height of the contraction wave of the several parts of the heart, including the total disappearance of a measurable contraction (vagus "arrest"), may be explained by variations in the conduction of the excitation process from one contractile unit to another. We must now inquire whether alterations in the frequency of contraction of the sinus or a part of the sinus can be similarly explained.

In Fig. 11 is presented an example of an apparently complete arrest of the whole heart. The ventricle was suspended in this experiment in such a manner as to permit the contractions of the sinus, auricle, and ventricle to be recorded in one curve. As a result of vagus excitation, all three of these parts seem to be arrested. If, however, the period of supposed total arrest is examined with great care, three regular but very faint elevations will be seen; these are the weakened contractions of the sinus.<sup>3</sup> A single record of these contractions, which are barely measurable, would convince no one. When however it is stated that such records have been repeatedly

<sup>1</sup> Compare ENGELMANN: Archiv f. d. ges. Physiol., 1896, lxii, p. 554.

<sup>2</sup> MUSKENS: *ibid.*, 1896, lxvi, p. 340.

<sup>3</sup> The engraver has very slightly sharpened these elevations in the curve (Fig. 11) in order to show their position clearly.

obtained in my experiments, the significance of these elevations must be conceded. I have observed every degree of lessening in the force of the sinus contraction down to this stage, in which the record trembles on the verge of complete obscurity. The arrest of the heart in these experiments is therefore only apparent. The excitation is still rhythmically discharged, but the resistance to its overflow upon the sinus, auricle, and ventricle is so much increased under the influence of the vagus that the change in form ceases to be measurable in the auricle and ventricle and is barely measurable in the sinus. Evidently should the conduction become still more difficult, even the sinus contraction would cease to be measurable, although contraction might still take place.

The change in rhythm just discussed is only apparent; the true rhythm of the heart, namely, the frequency with which the excitation wave is discharged in the sinus, has been unaltered. But we have to deal also with changes in the true rhythm. It has already been shown that the true rhythm may be increased or diminished by vagus action.

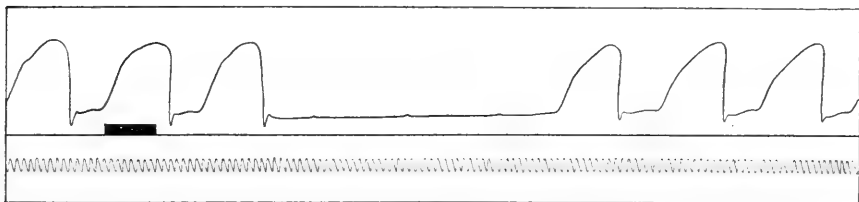


FIGURE 11. Three-fifths the original size. The ventricle was suspended in such a way that the contractions of the sinus, auricle, and ventricle are written in one curve. The black band in the line below marks the duration of the vagus excitation. The time is in tenths of seconds.

An additional example may be cited. In a *R. catesbeiana* the movements of the auricle and ventricle were recorded in one curve and the movements of the sinus in another. On excitation of the vagus the auricle and ventricle ceased to record, while the sinus beat with increased frequency. These cases can be readily explained by changes in the conducting power.<sup>1</sup> The number of excitation waves discharged from the sinus must depend largely on the resistance to the discharge. If the resistance is great, the threshold value will be high, and the discharge relatively infrequent; if the resistance is slight, the threshold value will be low and the discharge relatively frequent.

<sup>1</sup> Compare ENGELMANN: *Archiv f. d. ges. Physiologie*, 1896, lxii, p. 552.

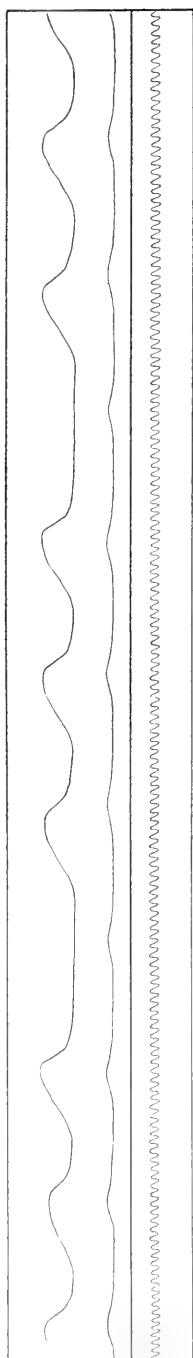


FIGURE 12. Five-sevenths the original size. The upper curve records the contractions of the ventricle; the lower, those of the auricle.

The probability of the vagus increasing or diminishing the resistance to the conduction of the excitation wave has been demonstrated above, and this action of the vagus suggests that the nerve may also regulate the resistance to the discharge of the excitation wave. The latter hypothesis is a simple and, to my mind, easily accepted explanation of the alterations in rhythm often observed when the vagi are stimulated; the threshold value of the excitation discharge is altered under vagus influence.

It should be stated here that an increase in the contraction interval also occurs apparently independently of the vagi in badly nourished hearts. Thus, in the exhausted frog's heart, especially after loss of blood, very interesting changes in the duration of the contraction interval may be observed.

In Fig. 12, the auricle (lower curve) beats with perfect regularity. The contractions of the ventricle are in groups of three. In each group, the auriculo-ventricular interval increases from nearly 0.7 to 1.1 secs. The intervals are 0.94, 1.07, dropped beat; 0.77, 1.01, 1.10, 1.14, dropped beat; 0.80, 1.01, 1.11, dropped beat.

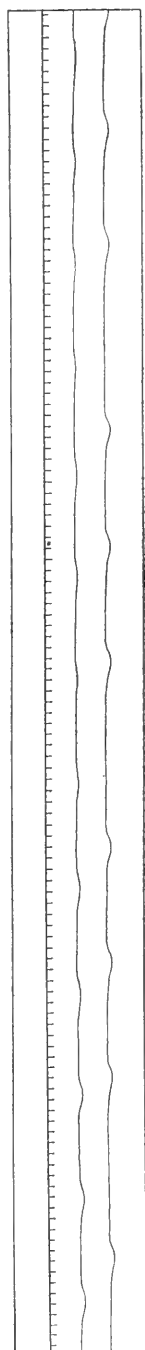
The sino-auricular interval is also sometimes lengthened in badly nourished hearts. Fig. 13 is an example. In this case the sinus beat with absolute regularity (lower curve), but the sino-auricular interval increased until the excitation wave was blocked entirely. The intervals are 1.38, 1.74, dropped beat; 1.16, 1.38, 1.56, dropped beat; 1.16, 1.38, 1.56, dropped beat; 1.16, 1.38, etc.

It seems highly probable that the periodic loss of one beat in the cases illustrated by Figs. 12 and 13 is a consequence of the

continuous increase of the contraction interval. Gaskell<sup>1</sup> and Engelmann<sup>2</sup> have shown that in the heart every contraction reduces the conducting power of the contracting part. In the record before us the auricle contracts with perfect rhythm. At each auricular contraction an excitation wave passes over the ventricle. On the arrival of the first excitation, the ventricle contracts. Its conducting power is lowered by this contraction. Recovery is slow because of imperfect nutrition. The next excitation from the regularly working auricle is delayed by the difficult conduction. Hence the second auriculo-ventricular interval is greater than the first. The excitation from the third auricular beat is delayed like the two preceding ones. The third auriculo-ventricular interval is longer than the second, as the second was longer than the first, for to the delay of the first interval is added the delay of the second. This process goes on. The auriculo-ventricular interval grows longer with each cardiac cycle, as the delay of each is added to the summed delays of its predecessors. Finally, the interval between the beat of the auricle and ventricle exceeds a certain limit, as in the case of vagus excitation (page 493), and a ventricular beat is dropped. The period is complete. The loss of this beat gives time for the conducting power to reach its former level. The next auriculo-ventricular interval is about the length of the first interval of the preceding series, and a new period begins.

This explanation of periodic pulses is of especial interest, for the reason that in badly nourished human hearts, for example in myocarditis and arterio-sclerosis, similar irregularities are not infrequent.<sup>3</sup> It is probable also that many of the

FIGURE 13. Two-thirds the original size. The upper curve records the contractions of the auricle; the lower, those of the sinus.



<sup>1</sup> GASKELL: *Journal of physiology*, 1883, iv, p. 97.

<sup>2</sup> ENGELMANN: *Archiv f. d. ges. Physiol.*, 1896, lxii, p. 543.

<sup>3</sup> Compare ENGELMANN: *Ibid.*, p. 553.

periodic groups of Luciani (recently studied by Oehrwall<sup>1</sup>) will be thus explained.

In conclusion I desire to express my grateful appreciation of the valuable criticism of Dr. H. P. Bowditch. I am also greatly indebted to Dr. W. T. Porter for assistance in the preparation of my paper for the press.

#### SUMMARY.

1. The sino-auricular and auriculo-ventricular contraction intervals are usually lengthened by vagus excitation; sometimes, however, they are diminished; the one may be increased, while the other is diminished. The vagus effect quickly reaches a maximum and then slowly decreases.

2. The interval between the contractions of different parts of the sinus is sometimes increased by vagus excitation, so that the different parts are dissociated and beat at measurably different times.

3. The force of contraction of the sinus and auricle is frequently diminished by vagus excitation.

4. The vagus does not diminish the force of ventricular contraction in the frog, except when the normal nutrition of the heart is disturbed by the loss of blood or otherwise.

5. The frequency of sinus contraction is usually diminished by the stimulation of the vagus; at times it is increased.

6. The various actions of the vagus nerve just enumerated, together with Bowditch's staircase, interference, and various forms of irregular pulse, can be readily explained by variations in the transmission of the cardiac excitation.

<sup>1</sup> OEHRWALL: Skandinav. Archiv für Physiologie, 1898, viii, p. 1.

## A NEW METHOD FOR THE STUDY OF THE ISOLATED MAMMALIAN HEART.

BY W. T. PORTER.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

THE isolation of the mammalian heart was first accomplished by H. N. Martin,<sup>1</sup> in the Biological Laboratory of the Johns Hopkins University. Normally warmed defibrinated blood entered the right auricle and right ventricle from a reservoir at normal venous pressure. The right ventricle pumped the blood through the lungs, where it was oxygenated, artificial respiration being maintained for that purpose. The pulmonary veins brought the arterialized blood to the left heart, and the left ventricle drove it into a tall tube tied into the aorta, whence the blood was returned to the venous reservoir. The height of the liquid column in this tube determined the pressure against which the ventricle worked, and this "arterial pressure" could be regulated at will.

The advantages of Martin's method are great. The heart is fully isolated from all other organs except the lungs, and works under conditions closely approximating the normal state. Indeed, for many purposes the original plan of Martin is superior to those since advocated. The chief objection to it has been the difficulty of securing blood enough. It is necessary to use dog's blood for the dog's heart, cat's blood for the heart of the cat, etc., and several animals have to be sacrificed in order to secure a sufficient quantity for one perfusion.

In 1890, Martin and Applegarth<sup>2</sup> published an important modification of the procedure just described. In the new method "all the branches of the aorta, except the coronary arteries, are ligated. The venæ cavæ are also ligated. In the aorta itself is placed a cannula, which is connected with a Mariotte's flask, raised a sufficient height above the organ. The defibrinated blood from the flask fills the

<sup>1</sup> MARTIN: Studies from the biological laboratory of the Johns Hopkins University, 1881, ii, p. 119.

<sup>2</sup> MARTIN and APPLGARTH: Studies from the biological laboratory of the Johns Hopkins University, 1890, iv, p. 275.

connecting tubes, the aorta, and the coronary arteries at a constant pressure, which, of course, is quite independent of the force and the frequency of the heart-beat. The blood taking the coronary circuit, on reaching the right auricle, proceeds to the corresponding ventricle, and from it through the lungs to the left auricle. This blood is, therefore, the only blood entering the cavities of the heart or passing through the lungs unless there be some inefficiency of the aortic semi-lunar valves. That the cavities of the heart are not distended with more blood is not found to influence the normal character of its beat, which continues rhythmically and forcibly for three or four hours."

Arnaud,<sup>1</sup> in 1891, injected defibrinated blood into the aorta of a rabbit the heart of which had ceased to beat, and saw co-ordinated contractions return.

The following year Hédon and Gilis<sup>2</sup> made similar injections in a dog and in an executed man, and secured co-ordinated beats.

Langendorff,<sup>3</sup> in 1895, modified the method of Martin and Applegarth by omitting the lungs, receiving the coronary blood from the right heart into a dish or beaker. The omission of the lungs permits the heart to be removed from the body, an advantage for certain purposes. Langendorff's modification is however open to the objection that the blood cannot be so satisfactorily oxygenated as when it passes through the lungs. Moreover, the removal of the heart prevents stimulation of the extrinsic cardiac nerves.

My own experiments on the isolated heart began nearly a year before the publication of Langendorff's first paper. They were at first directed to the discovery of a method by which the warm-blooded heart could be maintained in rhythmic, forcible contraction by thoroughly oxygenated blood supplied in the normal way, namely, through the right auricle to the right ventricle, thence to the left auricle and left ventricle, and so to the right auricle again. This is not the place to speak of the many devices which were employed one after the other in the attempt to secure a really satisfactory oxygenation of the blood. It is enough to state that none of these devices succeeded in thoroughly oxygenating in a sufficiently short

<sup>1</sup> ARNAUD: *Archives de physiologie*, 1891, p. 396.

<sup>2</sup> HÉDON and GILIS: *C. r. de la soc. de biologie*, Paris, 1892, p. 760.

<sup>3</sup> LANGENDORFF: *Archiv f. d. ges. Physiol.*, 1895, lxi, p. 292. Langendorff's modification has recently been employed in altered form by RÜMKE: *Geneeskundige Bladen uit Kliniek en Laboratorium*, Harlem, 1897, (iv) x, p. 201.



time the quantity of blood required for the successful perfusion of the warm-blooded heart. The attempt was therefore temporarily given over and the method of Martin and Applegarth employed.

My first arrangement of Martin and Applegarth's method agreed with Langendorff's modification in omitting the lungs, but differed from Langendorff's in many other respects. All the branches of the aorta except the coronary arteries were tied. The aorta was kept filled with blood from a reservoir at constant pressure. The semilunar valves being closed by the constant high aortic pressure, the blood was forced through the coronary arteries into the right heart; a very small quantity entered the left heart through the vessels of Thebesius. The force and frequency of the contractions of the left ventricle were recorded by a Hürthle manometer connected with a tube passed into the left ventricle through the left auricular appendix and mitral valve. The blood flowing through the coronary vessels into the right heart escaped through the pulmonary artery on to a registering apparatus, so that the volume of the coronary circulation, excepting the very small quantity reaching the left heart through the vessels of Thebesius, was recorded.

With this method the fact that stimulation of the vagus diminishes the flow through the coronary arteries was discovered,<sup>1</sup> an experiment recently repeated by Maas<sup>2</sup> in Langendorff's laboratory. Curves showing simultaneously the intraventricular pressure and the diminution in the volume of the coronary circulation in vagus excitation, obtained by this method, were shown to the American Physiological Society at its meeting in Boston in December, 1896. One of the curves is to be found in this *Journal*, 1898, i, p. 160. Another was published in the *American Text-book of Physiology*, 1896, p. 453, to illustrate the influence of the vagus on the frequency of ventricular contraction.<sup>3</sup> This is the first instance in which a graphic record of the volume of the coronary circulation has been obtained; and the first in which the intraventricular pressure in the isolated heart has been recorded.

With this method also the relation of the volume of the coronary

<sup>1</sup> PORTER: *Boston med. and surg. journal*, 1896, cxxxiv, p. 39.

<sup>2</sup> MAAS: *Archiv f. d. ges. Physiol.*, 1898, lxxi, p. 399.

<sup>3</sup> In reproducing this curve, the line recording the volume of the coronary circulation was cut out because unnecessary to the illustration of the vagus action on the frequency of the heart; this curve and the one published in the *American Journal of Physiology* were from the same experiment, March 26, 1896.

circulation to the frequency and force of the ventricular contraction in the isolated heart of the cat was studied. Curves showing the main result of this investigation were published in the *American Text-book of Physiology*, 1896, p. 476. A detailed account is to be found in the *Journal of Experimental Medicine*.<sup>1</sup>

In the method last described the animal and the recording apparatus were placed in a huge warm chamber kept at a constant temperature. After a time, I discarded the warm chamber and designed in its stead the apparatus afterwards used by Miss Hyde in her study of the effect of distention of the ventricle on the flow of blood through the walls of the heart. The new method is described in her communication in this *Journal*.<sup>2</sup>

Meanwhile, I had found that any part of the ventricle of the dog's heart, even the ganglion free apex, will beat for hours if supplied with defibrinated dog's blood through its nutrient artery. By this means the whole ventricle, as well as the apex of the ventricle, was for the first time fully isolated from the rest of the heart and kept in powerful, rhythmic, long-continued contraction.<sup>3</sup> This method has now been constantly used in this laboratory during fourteen months, and can be highly commended. Dogs are usually employed. The animal is anæsthetized with ether, bled from the left carotid artery, the blood defibrinated, and filtered through glass wool. Meanwhile, warm 0.8 per cent sodium chloride solution is allowed to flow into the right jugular vein. After a short interval, the dog is bled again from the carotid artery, and the blood defibrinated as before. The heart is now rapidly removed and placed still beating in a beaker of warm saline solution. Often the beats are so vigorous that the heart with each ventricular systole springs more than an inch from the bottom of the beaker. Thus the organ is self-cleansed from blood. A glass cannula is now tied into the coronary artery supplying the area the contractions of which are to be studied, and the part of the heart wall supplied by the artery is cut out. The cannula bearing the attached ventricular segment is filled with defibrinated blood and joined to a glass tube passing through the rubber stopper of a small glass chamber. A small adjustable clamp sup-

<sup>1</sup> MAGRATH and KENNEDY: *Journal of experimental medicine*, 1897, ii, p. 13.

<sup>2</sup> Miss I. H. HYDE: *This journal*, 1898, i, p. 215.

<sup>3</sup> PORTER: *Journal of experimental medicine*, 1897, ii, p. 391; preliminary account in *Journal of the Boston Society of Medical Sciences*, 1897, i, issued March 10, 1897.

ports the upper margin of the piece of heart to keep its weight from dragging on the nutrient artery. The chamber is provided with a thermometer. The neck of the chamber passes through a rubber stopper in the floor of a galvanized iron water tank, the sides of which rise above the top of the chamber. A wire attached by a hook to the lower end of the piece of ventricle passes through the neck of the chamber and is fastened to a lever of the second class, the writing-point of which traces the contraction curve, usually magnified seven times, upon a Baltzar drum. Within the water tank also is placed a litre flask filled with defibrinated blood. The contents of this flask are kept at any desired pressure by means of a pressure-bottle. The blood passes from the blood-flask to the heart-chamber and through the cannula in the coronary artery into the ventricular segment, from which it escapes through the severed veins into the lower part of the chamber and runs out into a tall beaker placed to receive it. The blood-flask, the heart-chamber, and the tubes connecting the two are surrounded by a large volume of water at any desired temperature. The water tank is thirty-six centimetres long, twenty-one broad, and twenty-five deep. A window in the front permits a view of the heart as it contracts.

The advantages of this method for studying the fundamental properties of cardiac muscle, the action of animal extracts and drugs upon the heart, and certain other problems, are very great. A large dog's heart affords two and sometimes three separate apex-preparations each with a nutrient artery large enough for a practicable cannula. Several preparations of the basal portion of the ventricle can also be obtained from the same heart. The experiment scarcely ever fails. The perfused piece seldom refuses to beat, and if it does another piece from the same heart can be used. The relatively very large quantity of perfusion fluid allows the circulation to continue for a long time; there is no troublesome turning of stopcocks at frequent intervals. Often the quantity of blood together with the small number of vessels to be supplied makes it unnecessary to perfuse the heart twice with the same blood. The preparation can be made with all care. There is no hurry. Even pieces left for more than an hour will usually beat when perfused. The long survival makes it possible to prolong experiments through most of the day, a fresh piece of ventricle being taken when the one in use wears out. This change of pieces we have found of value in testing the effect of poisons and animal extracts.

Such are the various methods of isolating the mammalian heart. At best, they leave much to be desired. They fail to realize the long-deferred hope that the mammalian heart shall be made to beat like the heart of the frog in a Williams apparatus, contracting for hours while fed on a simple perfusion fluid. This result is reached by the following procedure.

It will be remembered that the great obstacle to the perfection of the methods of isolating the mammalian heart has been the difficulty of properly oxygenating the nutrient blood. Oehrwall<sup>1</sup> has shown that even the batrachian heart contracts much more powerfully when surrounded with pure oxygen. Since the publication of Oehrwall's paper many plans for the use of oxygen in the isolation of the warm-blooded heart have been tried by the present writer without avail. In every instance the mechanical difficulties of keeping considerable quantities of blood thoroughly oxygenated have been too great. Not until the discovery that the mammalian heart would beat with a blood-supply much less than is ordinarily thought to be essential,<sup>2</sup> and the further discovery that this relatively small quantity may be effectively supplied to the heart muscle through the veins of Thebesius and the coronary veins<sup>3</sup> did the problem seem once more practicable. On returning to the attack, I determined to feed the heart through these veins in an atmosphere of oxygen, and, if this were not enough, to increase the oxygen pressure, in the hope of thereby facilitating oxidation in the manner taught by Haldane.<sup>4</sup>

The following experiment was accordingly performed.

*May 2, 1898.* A cat was bled and the blood defibrinated and filtered through glass wool. Cannulas were tied into the right auricular appendix, the pulmonary artery, and the aorta. The cannula in the right auricular appendix led through a Williams valve to a small reservoir of blood. The pulmonary and aortic cannulas were each connected with glass tubes which rose to a short distance above the blood-reservoir and then turned to discharge their contents into the reservoir itself. All the heart vessels except the two arteries mentioned were ligated. The arrangement therefore was closely similar to that of the frog's heart in a Williams apparatus. The heart with its several tubes was now placed in a strong glass cylinder immersed in warm water. The top of the cylinder was provided with a stout brass cap

<sup>1</sup> OEHRWALL: *Archiv für Physiologie*, 1893, Suppl. Bd., p. 40.

<sup>2</sup> MAGRATH and KENNEDY: *Journal of experimental medicine*, 1897, ii, p. 13.

<sup>3</sup> PRATT: *This journal*, 1898, i, p. 86.

<sup>4</sup> HALDANE: *Journal of physiology*, 1895, xviii, p. 211.

perforated by two tubes. One was a T-tube the side branch of which led to a large metal cylinder containing oxygen under high pressure, while the other branch was provided with a stop-cock opening into the atmospheric air. The second tube led to a pressure gauge. So soon as the oxygen pressure began to rise, the heart, which had ceased to beat, began to contract with great vigor. Surrounding the heart with oxygen even at the pressure of the atmosphere was distinctly helpful, but the contractions became decidedly stronger and more frequent as the oxygen pressure rose. A pressure of about two atmospheres was that ordinarily employed, but as high as four atmospheres was occasionally tried. The blood coursed from the reservoir into the right side of the heart. Each beat of the right ventricle drove blood in a stream through the tube in the pulmonary artery back into the reservoir. The Williams valve prevented regurgitation from the right heart. The heart muscle was nourished through the veins of Thebesius and the coronary veins. A small quantity of blood found its way through foramina Thebesii into the left auricle and ventricle, whence it was pumped by the latter out through the aorta. The vigorous beating of the heart continued from early in the morning until late in the afternoon, when the experiment was broken off. The contractions were vigorous also at room temperature.

Two conclusions may be drawn from this experiment: —

(1) An atmosphere of oxygen is of advantage in maintaining the contractions of the isolated mammalian heart.

(2) A heart fed simply through the veins of Thebesius and the coronary veins will maintain strong, rhythmic contractions for many hours if supplied with oxygen at high tension.

The first thought suggested by these statements is whether the mammalian heart, like the frog's heart, will beat when fed on serum alone, provided that a sufficient supply of oxygen is furnished. The experiment was accordingly repeated on other hearts, but the blood was replaced by serum obtained by centrifugalizing defibrinated blood. As was expected, the absence of corpuscles was readily borne by the heart. Continued rhythmic contractions were obtained with the serum alone, so soon as the oxygen tension rose to about two atmospheres.

It follows that *the mammalian heart fed through the vessels of Thebesius and the coronary veins with blood-serum alone will maintain rhythmical contractions for hours when surrounded by oxygen at high tension.*

The ease with which this remarkable result was obtained encouraged the hope that even isolated pieces of the ventricle would beat if

fed with serum through a branch of the coronary artery. It was *a priori* almost certain that this would be the case, were the piece of ventricle supplied with serum at the normal blood-pressure. But to force serum through a coronary artery at the normal pressure requires a pressure-apparatus difficult of control in an extrinsic pressure of two atmospheres. Even were this difficulty overcome, the rate of flow through a piece of ventricle fed at fairly high pressure is rapid and a large volume of serum would be required. Now, a sufficiently large volume of serum cannot be obtained from a single animal, and it is somewhat disadvantageous to use the blood of other animals, even of the same species. It seemed best, then, to attempt perfusion at a very low arterial pressure, trusting that even this slight driving force would carry serum enough through the capillaries to produce and maintain contractions. The complete success of this undertaking is shown in the following experiment.

*June 17, 1898.* A cat, anæsthetized with ether, was bled from the left carotid artery, the blood defibrinated, diluted one-half with 0.8 per cent NaCl solution, and the serum separated in a centrifugal machine. An hour after the heart had ceased to beat it was removed from the chest, a cannula tied into the ramus descendens of the left coronary artery, and the part of the ventricle supplied by this branch cut away. The cannula was joined to a vessel containing 50 c.c. of the cat's serum, and placed in a glass cylinder connected with an oxygen reservoir. The height of the column of serum above the piece of ventricle was about 25 centimetres. The flow was approximately at the rate of one drop per second. The temperature was that of the room, about 25°C. The oxygen pressure was now raised to nearly two atmospheres. In a very few minutes the piece of ventricle began to beat with regularity and force, and these strong and rhythmical contractions continued so long as the supply of serum was kept up. When the serum ceased to pass, the ventricle ceased to beat.<sup>1</sup>

This experiment permits the further conclusion that *even isolated portions of the mammalian ventricle supplied through their nutrient arteries with a small quantity of serum at very low pressure will maintain rhythmical, long-continued, forceful contractions when surrounded by oxygen at high tension.*

<sup>1</sup> Similar results have been since attained with the isolated apex of the dog's heart.

## INDEX TO VOL. I.

---

- A**BSORPTION, intestinal, 411.  
 Activity, daily, methods of recording, 40.  
 —, variations produced by alcohol, barometric changes, and diet, 40.  
**ALBRO, ALICE H.** See **CHITTENDEN, R. H.**, and **ALICE H. ALBRO**, 307.  
 Albumose, excretion by kidneys, 274.  
 —, physiological action, 266.  
 Alcohol, effect on distemper, xv.  
 —, effect on voluntary muscular power in fatigue, xv.  
 —, effect on young dogs, xv.  
 Alcoholic beverages, influence on digestion, 184.  
 American Physiological Society, Proceedings, iii.  
 Amyolytic power of saliva, variations, iii.  
 Anæsthesia, by ether in rectum, viii.  
 Aorta, blood currents in, xiv.  
 Attention, determination of a constant of, 283.
- B**ANCROFT, F. W. The venomotor nerves of the hind limb, 477.  
**BARROWS, F. W.** The effect of inanition on the structure of nerve cells, xiv.  
 Bile, chemical reaction, 317.  
 —, influence on pancreatic proteolysis, 307.  
 Biological problems of to-day, xv.  
 Blood-pressure, influenced by albumose and peptone, 275.  
 Borax, its influence on nutrition, 1.  
 Boric acid, its influence on nutrition, 1.  
 Brain-circulation, influenced by high arterial pressures, 57.  
**BROWN, E. W.** Notes on *Cetraria islandica* (*Iceland moss*), 455.  
**BUDGETT, S. P.** On the similarity of structural changes produced by lack of oxygen and certain poisons, 210.
- C**ALCIUM SALTS, solubility, 423.  
 Cane-sugar, inversion in stomach, 277.  
**CANNON, W. B.** See **MOSER** and **CANNON**, xii.  
**CANNON, W. B.** The movements of the stomach, studied by means of the Röntgen rays, 359, xiii.  
**CANNON, W. B.**, and **A. MOSER.** The movements of the food in the œsophagus, 435.  
 Cardiopneumatic movements, 117.  
 Cathartics, 411.  
*Cetraria islandica* (*Iceland moss*), 455.  
**CHITTENDEN, R. H.** Variations in the amyolytic power of saliva and their relation to the chemical composition of the secretion, iii.  
**CHITTENDEN, R. H.**, and **ALICE H. ALBRO.** The influence of bile and bile salts on pancreatic proteolysis, 307.  
**CHITTENDEN, R. H.**, and **W. J. GIES.** The influence of borax and boric acid upon nutrition, with special reference to proteid metabolism, 1.  
**CHITTENDEN, R. H.**, **L. B. MENDEL**, and **H. C. JACKSON.** A further study of the influence of alcohol and alcoholic drinks upon digestion, with special reference to secretion, 164.  
**CHITTENDEN, R. H.**, **L. B. MENDEL**, and **H. E. McDERMOTT.** Papain-proteolysis, with some observations on the physiological action of the products formed, 255.  
**CHITTENDEN, R. H.**, and **A. N. RICHARDS.** Variations in the amyolytic power and chemical composition of human mixed saliva, 461.  
**CLARK, G. P.** On certain characteristics of the pressure sensations of the human skin, 346, xi.  
**CLEGHORN, A.** The reinforcement of voluntary muscular contractions, 336.

Coagulation of blood influenced by albumose and peptone, 269.

Color vision, xv.

Contraction, static, methods of determining, 284.

Coronary circulation, volume of, method of recording, 215.

Coronary pulse-wave, 152.

Coronary veins, nourish the heart, 86.

Coronary vessels compressed in systole, 145.

CUNNINGHAM, R. II. The restoration of coordinated volitional movement after nerve "crossing," 239.

CUSHNY, A. R. See WALLACE and CUSHNY, 411.

**D**EGLUTITION, 435, xii.

—, studied with Röntgen rays, 435, xii.

Distemper, affected by alcohol, xv.

Depressor-nerve in guinea-pig, 393.

Development, affected by alcohol, xv.

Diabetes, 395.

Digestion by papain, 255.

Digestion, influenced by alcohol, 164.

Digestion of cane-sugar, 277.

Dynamograph, 284.

**E**AR, function in fishes, 128.

Electrodynamometer, 106.

Ether-anæsthesia by the rectum, viii.

**F**ATIGUE, affected by alcohol, xv.

Fatty degeneration, metabolism in, v.

FERRIS, S. J., and G. Lusk. The gastric inversion of cane-sugar by hydrochloric acid, 277.

Fibrillary contractions of heart, 71, 99.

Friction-machine, 294.

Fungi, composition and nutritive value, 225.

**G**ALVANOMETER, Rowland, 106.

Gastric cannula, 191.

GIES, J. See CHITTENDEN and GIES, I.

GREENE, C. W. On the relation between the external stimulus applied to a nerve and the resulting nerve impulse as measured by the action current, 104.

Guinea-pig, cardiac nerves in, 383.

**H**ALLOCK, W. (with F. S. MUCKEY). The action of the larynx in the production of voice, vi.

HARRINGTON, D. W. Contributions to the physiology of the cardiac nerves in the guinea-pig, 383.

Heart, conduction of excitation wave, 502.

—, contraction interval, 493.

—, force, 497.

—, distention influences intramural flow, 215.

—, fibrillary contractions, 71.

—, frequency, 500.

—, influenced by vagus nerve, 486.

—, intramural circulation increased by systole, 157.

—, mammalian, method of isolating, 93, 215, 511.

—, nerves in guinea-pig, 383.

—, nutrition through vessels of Thebesius and coronary veins, 86, 516.

—, oxygen at high tension replaces red corpuscles, 516.

HODGE, C. F. Influence of alcohol upon voluntary muscular power in conditions of fatigue, xv.

—, Influence of alcohol upon the young in dogs, and upon the severity of an attack of distemper, xv.

HOWELL, W. H. The influence of high arterial pressures upon the blood-flow through the brain, 57.

HYDE, IDA H. The effect of distention of the ventricle on the flow of blood through the walls of the heart, 215.

Hydrochloric acid inverts cane-sugar, 277.

**I**CELAND moss, 455.

Inanition, effect on nerve cells, xiv.

Intestinal absorption, 411.

**J**ACKSON, H. C. See CHITTENDEN, MENDEL, and JACKSON, 164.

**K**YNURENIC acid, excretion of, xv.

**L**ARYNX, action in voice-production, vi.

Lateral line in fishes, function, 128.

LEE, F. S. The functions of the ear and the lateral line in fishes, 128.

Lichenin, 456.



LOEB, J. The biological problems of today : physiology, xv.

Lung, redistention after collapse, ix.

LUSK, G. See FERRIS and LUSK, 277.

——. See REILLY, NOLAN, and LUSK, 395.

——. On metabolism in fatty degeneration, v.

MATHEWS, A. A contribution to the chemistry of cytological staining, 445.

MCDERMOTT, H. E. See CHITTENDEN, MENDEL, and MCDERMOTT, 255.

MELTZER, S. J. A new pleural cannula *in situ*, xv.

——. A simple method for the redistention of the collapsed lung, ix.

——. Ether-anæsthesia by the rectum, viii.

——. On the nature of the cardiopneumatic movements, 117.

MENDEL, L. B. See CHITTENDEN, MENDEL, and JACKSON, 164.

——. See CHITTENDEN, MENDEL, and MCDERMOTT, 255.

——. Some experiments on the excretion of kynurenic acid, xv.

——. The chemical composition and nutritive value of some edible American fungi, 225.

Mental activity, measurement through muscular activity, 283.

Metabolism, in fasting animals, 395.

——, in fatty degeneration, v.

——, in phlorhizin diabetes, 395.

MOSER, A. See CANNON and MOSER, 435.

MOSER, A., and W. B. CANNON. The movement of food in deglutition, xii.

MUCKEY, F. S. See HALLOCK and MUCKEY, vi.

Muscle, active relaxation, 343.

Muscular contractions, reinforcement, 336.

Muscular power, affected by alcohol, xv.

Mushrooms, composition and nutritive value, 225.

MUSKENS, L. J. J. An analysis of the action of the vagus nerve on the heart, 486.

NERVE cells, affected by inanition, xiv.

——. Nerve-crossing, restoration of movement after, 239.

Nerve impulse, relation to external stimulus, 104.

Nerves of veins, 477.

NOLAN, F. W. See REILLY, NOLAN, and LUSK, 395.

ŒSOPHAGUS, movements of food in, 435.

Oxygen, lack of, causes changes in protozoa, 210.

PANCREATIC proteolysis, influenced by bile and bile salts, 307.

Papain-proteolysis, 255.

PATTEN, W. A basis for a theory of color vision, xv.

Peptone, physiological action, 266.

——, excretion, 274.

Phlorhizin diabetes, 395.

Pleural cannula, xv.

PORTER, W. T. A new method for the study of the isolated mammalian heart, 511.

——. New experiments on the mammalian heart, xiv.

——. The influence of the heart-beat on the flow of blood through the walls of the heart, 145.

——. The recovery of the heart from fibrillary contractions, 71.

PRATT, F. H. The nutrition of the heart through the vessels of Thebesius and the coronary veins, 86.

Pressure sensations of human skin, 346, xi.

Proceedings of the American Physiological Society, iii.

Proteid, its constitution, 409.

Proteid metabolism, influenced by borax and boric acid, 1.

Proteolysis, influenced by bile, 307.

Protozoa, structure changed by lack of oxygen and by certain poisons, 210.

Pulse, irregularities explained, 508.

RECTUM, anæsthesia by ether, viii.

——. Reinforcement of voluntary muscular contractions, 336.

REILLY, F. H., F. W. NOLAN, and G. LUSK.

Phlorhizin diabetes in dogs, 395.

RICHARDS, A. N. See CHITTENDEN and RICHARDS, 461.

SALIVA, amylolytic power, 461, iii.

——, chemical reaction, 463.

——, composition, 461, iii.

——, secretion, influenced by alcohol, 164.

——, submaxillary of dog, composition, 166.

——, variations in amylolytic power, 461, iii.

Salivary digestion in stomach, 379.

Skin, pressure sensations, 346, xi.

Staining, chemistry of, 445.

STEWART, C. C. Variations in daily activity produced by alcohol and by changes in barometric pressure and diet, with a description of recording methods, 40.

Stomach, absorption of alcohol from, 205.

—, anatomy in cat, 364.

—, digestion of cane-sugar, 277.

—, duration of digestion, 202.

—, inhibited by emotions, 380.

—-movements, 359, xiii.

—, reaction of contents, 378.

—, salivary digestion in, 379.

Strontium elimination, 83.

Sugar production, 402.

THEBESIOUS, vessels of, anatomy, 87.  
Toricular pulse, 68.

VAGUS-NERVE in guinea-pig, 384.

—, influence on heart, 486.

—, method of stimulating, 488.

Vasomotor nerves. See Venomotor.

Veins, nerves of, 477.

Veins of Thebesius, anatomy, 87.

—, nourish the heart, 86, 516.

Venomotor nerves of hind limb, 477.

Ventricle, distention checks flow of blood through walls of heart, 215.

Voice production, vi.

Vomiting, 373.

WALLACE, G. B., and A. R. CUSHNY.  
On intestinal absorption and the saline cathartics, 411.

WELCH, JEANNETTE C. On the measurement of mental activity through muscular activity, and the determination of a constant of attention, 283.

WOOD, H. C., Jr. Notes on the elimination of strontium, 83.

PROCEEDINGS OF THE AMERICAN PHYSIO-  
LOGICAL SOCIETY.

TENTH ANNUAL MEETING.

CORNELL UNIVERSITY, DECEMBER 28 and 29, 1897.



## PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

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### VARIATIONS IN THE AMYLOLYTIC POWER OF SALIVA AND THEIR RELATION TO THE CHEMICAL COMPOSITION OF THE SECRETION.

By R. H. CHITTENDEN.

IN some experiments conducted in 1882<sup>1</sup> an attempt was made to ascertain whether there is any definite relationship between the amylolytic power of human saliva and its degree of alkalinity. In the experiments then recorded, alkalinity was determined by titration with a standard acid, using cochineal as an indicator, while amylolytic power was estimated by determining the quantity of sugar formed from a definite amount of starch under given conditions. The results led to the conclusion that such variations in amylolytic power as saliva ordinarily shows, are not associated with corresponding variations in the degree of alkalinity.

In a recent paper by Hofbauer,<sup>2</sup> the above results are referred to with the statement that they constitute the only data recorded bearing on the amylolytic power of human saliva at different periods of the day. This statement, however, is quite misleading, for in our paper it is distinctly stated that "the saliva was collected generally an hour or two after breakfast," no attempt having been made to ascertain variations in amylolytic power for different periods of the day; indeed, in practically all of our experiments at that time, the saliva was collected at a convenient period after breakfast. The average alkalinity expressed in terms of sodium carbonate of fifty-one samples of saliva was found by the above method to be 0.08 per cent, the extremes being 0.052-0.163. We would now call attention to the fact that human saliva, while ordinarily alkaline to litmus

<sup>1</sup> CHITTENDEN and ELY: On the alkalinity and diastatic power of human saliva. *American chemical journal*, 1883, iv, p. 329.

<sup>2</sup> HOFBAUER, L.: Tägliche Schwankungen der Eigenschaften des Speichels. *Archiv f. die ges. Physiol.*, 1897, lxxv, p. 503.

or lacmoid, is almost invariably acid to phenolphthalëin, hence such alkalinity as it possesses, is due not to sodium carbonate but mainly to alkaline phosphates, acid phosphates being likewise present. Experiments made in our laboratory by Mr. A. N. Richards show that human mixed saliva, using lacmoid as an indicator, requires on an average 0.7 milligram  $\text{H}_2\text{SO}_4$  to neutralize the alkalinity of 1 gram of the secretion. Expressed in terms of sodium carbonate, this would be equal to an alkalinity of 0.14 per cent. With phenolphthalëin as an indicator, on the other hand, 1 gram of saliva requires on an average 0.06 milligram  $\text{NaOH}$  to neutralize the acid salts present. It has also been found that the alkalinity as indicated by lacmoid and the acidity as indicated by phenolphthalëin are both noticeably greater in the saliva collected before breakfast than in the secretion collected after breakfast. Further, in conformity with Hofbauer's results, we find, as a rule, that the amylolytic power of saliva coming from glands which have been in a state of rest for some time, *i. e.*, collected before breakfast, is greater than that secreted an hour after breakfast. We are not inclined, however, to consider that the increased amylolytic power of saliva secreted before breakfast, for example, is to be attributed directly to the increased alkalinity, for occasional results show that amylolysis may be more pronounced with saliva having a comparatively low degree of alkalinity. The true explanation is to be found in the greater concentration of the secretion coming from the glands which have been in a state of inactivity; *i. e.*, such secretion contains a larger amount of solid matter with a corresponding increase in the proportion of amylolytic enzyme, etc. The results of a single experiment may be cited: —

Date.	Time. <sup>1</sup>	Alkalinity. <sup>2</sup>	Amylolytic power. <sup>3</sup>	Solids.	Organic matter.	Salts.
Nov. 24.	7.10-7.30 A.M.	0.163%	651.0	0.86%	0.58%	0.28%
"	9.00-9.30 "	0.112	615.6	0.51	0.30	0.21

Numerous results similar to the above testify to the truth of the foregoing statement. Somewhat noticeable also is the influence of

<sup>1</sup> Before and after breakfast.

<sup>2</sup> Determined by  $\frac{1}{10}$  normal  $\text{H}_2\text{SO}_4$  with lacmoid as an indicator and expressed as sodium carbonate.

<sup>3</sup> Expressed as milligrams of maltose formed from 1 gram of starch.

different stimuli upon the amylolytic power and chemical composition of human saliva. Experiments have been made with ether and chloroform vapor, alcohol, whiskey, and gin, the secretion obtained under their influence being compared with that resulting from mechanical stimulation, etc. The results thus far obtained tend to show that the above agents cause the secretion of a fluid richer in amylolytic enzyme and having a higher content of solid matter. The details of the experiments will be published in the next number of this Journal.

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## ON METABOLISM IN FATTY DEGENERATION.

By GRAHAM LUSK.

MANY years ago Voit declared his belief in a preliminary cleavage of the proteid molecule within the organism into a nitrogenous portion and a non-nitrogenous portion, which were subsequently burned within the cells, often at different times. To the non-nitrogenous portion belonged the sugar of the starving diabetic, and it likewise furnished fat in fatty degeneration. Through the subcutaneous injection of phlorhizin in dogs a ratio of sugar to nitrogen as 3.75 is to 1 has been established in the laboratory of the writer. This signifies that the proteid molecule may yield 60 per cent of dextrose. Accompanying this intense form of diabetes may be seen in the starving dog a rise of 450 per cent in the proteid decomposition, an effect probably due to the non-combustion of the sugar produced. The only case parallel to this in the extent of its proteid decomposition lies in phosphorus poisoning, where a similar increase is present. The question arises, is not this high proteid metabolism in phosphorus poisoning likewise due to the non-burning of the sugars, consequent upon their quantitative conversion into fat? In other words, may not the 60 grams of dextrose obtainable from every 100 grams of proteid be converted into fat in cases of acute fatty degeneration?

In a first experiment upon a diabetic dog the ratio in the urine was found to be Dextrose: Nitrogen = 3.75: 1. During the administration of the phlorhizin, phosphorus oil was also given, with the idea of reducing possibly the sugar in the urine by means of its conversion into fat. No decrease in the sugar followed, although the dog died with every symptom of phosphorus poisoning. This experiment,

however, does not disprove the idea that in fatty degeneration the sugar from proteid is converted into fat, for the phlorhizin may have protected the sugar immediately upon its formation from any further change. A second experiment made the subject clearer. A starving dog was poisoned with phosphorus; all the symptoms, including a high rise in proteid metabolism, were manifest. Under these circumstances, if proteid sugar is being converted into fat there should be no sugar present in the body. Now, the action of phlorhizin is first to sweep the body clear of sugar, as is indicated by the high ratio of sugar to nitrogen observed always on the first day of phlorhizin administration, even after long fasting. If now in the dog poisoned with phosphorus no sugar was present and we administered phlorhizin, no excess of sugar should be eliminated; only that belonging to the proteid decomposition for the time being should be eliminated. The result obtained conformed with this theoretical expectation. The ratio in the urine was Dextrose: Nitrogen = 3.65: 1. This indicates that in phosphorus poisoning there is no sugar present in the dog. Either one of two conditions may here be possible: either the sugar is burned as soon as formed, or it is converted into another substance. That it is immediately burned is improbable on account of the high proteid metabolism;—its burning would reduce proteid metabolism. The sugar must, therefore, have been converted into another substance or into fat. It seems reasonable to conclude that in acute fatty metamorphosis of the cell the dextrose formed from proteid in the cytoplasm may be quantitatively converted into fat.

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## THE ACTION OF THE LARYNX IN THE PRODUCTION OF VOICE.

By W. HALLOCK (with F. S. MUCKEY).

THE organ of voice-production is essentially a string, not a reed instrument. The two fundamental reasons for this conclusion are: first, the agencies for the control of pitch are the agencies that control the pitch of a string, namely, tension, length, and weight; secondly, the quality of the tone produced is the quality of the tone of a string.

Voice-production and voice-modification (articulation) are managed by distinct, independent sets of muscles, the former by the intrinsic



laryngeal muscles, the latter by the extrinsic muscles; and neither set should be permitted to usurp or interfere with the functions of the other.

In the correct production of voice there should be no registers. The three agencies for the control of pitch are mediated by the intrinsic laryngeal muscles only. They should act simultaneously, independently, evenly, and gradually, and produce a smooth and continuous rise in pitch from the lowest tone to the highest, the action and operation of the larynx being the same throughout. If the extrinsic muscles are allowed to come into action and pull upon the larynx, the latter is distorted and the delicate action of the arytenoid cartilages is absolutely blocked. It then becomes necessary to rely entirely on change of tension to control pitch, and of the three factors this is the most difficult of control, because the pitch is directly proportional to only the square root of the tension of the cords, whereas it is inversely proportional to the length and weight. Under these conditions registers arise, owing to the imperfect coöperation of and coördination between the intrinsic and extrinsic muscles, and the cords are seriously strained by the high tension to which they must be submitted in the effort to produce the high tones. This abnormal strain results in impairment of the muscle-structure, and then in faulty approximation of the vocal bands, with all the evil consequences thereof. The most pernicious of all habits in voice-production is this of permitting the large and powerful extrinsic muscles to usurp the duties of the delicate intrinsic muscles and prevent their action, while unable themselves to accomplish the same results.

The classic investigations of Helmholtz, König, and many others, have proved that in the human voice the sound consists of a fundamental or pitch tone, accompanied by one or more of a series of overtones, the quality of the voice being dependent upon the latter. By photographing the movements of sensitive flames we have been able to analyze tones and thus to verify completely the general correctness of the visual and oral observations of Helmholtz and König. Our photographs give an impersonal impartial record of the "string overtones" in the voice, and their modification of quality, not only in different voices, but in different vowel sounds in the same voice.

In order to reinforce a tone, a cavity must have a fixed size, shape, and opening. The vibrations must be able to pass in as well as out at the opening. Reinforcement by chest-resonance is impossible, for two reasons especially: the chest is a cavity of varying size even

during a single breath, and it is essentially a closed cavity. The air in the chest may, and does vibrate, — so it does in the wind-box of an organ, — but these vibrations cannot reinforce the tone produced externally. The antra and sinuses are also useless for resonant reinforcement.

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## DEMONSTRATION: ETHER-ANÆSTHESIA BY THE RECTUM.

BY S. J. MELTZER.

A SMALL bottle half filled with ether, and closed with a cork perforated by a glass tube, was placed in a water-bath at a temperature above the boiling point of ether. The ether vapor generated was led into the rectum by means of a metal tube provided with a number of side openings, besides an aperture at the end, and connected with the ether bottle by means of rubber tubing. As ether boils at a point below the temperature of the body (about  $35^{\circ}\text{C.}$ ), the introduced vapor remains in the intestinal canal in a gaseous state, and is there readily absorbed. The rate of absorption in this place is by no means comparable with that of the absorption of ether by the lungs. The absorption, however, is greatly facilitated by an increase of the intra-intestinal pressure, which can be easily accomplished by increasing the temperature of the water-bath, and thus introducing rapidly large quantities of ether vapor into the intestines. It must be borne in mind that the favorable as well as the dangerous state of anæsthesia depends upon the amount of ether present at one time in the blood, and this depends not only upon the rate of absorption, but also upon the rate of the excretion from the body. Ether is always excreted through the lungs. If too large quantities of ether vapor are rapidly thrown into the intestines, not only will absorption be increased, but the enormously developing meteorism may seriously impair the respiration, and considerably diminish the excretion of the ether, and thus cause death. On the other hand, the excretion of ether by the lungs is apparently more completely accomplished when the vapor is introduced into the rectum than when inhaled by the lungs, as in the latter case the ether has to be exhaled into an atmosphere already saturated with ether. If the temperature of the water is not too high, and if care is taken to remove frequently the ether bottle from the water-bath, the ether anæsthesia by the rectum is a safe and convenient method for certain laboratory purposes. Thus, a rab-

bit can be narcotized in a few minutes, and can be kept in a state of anæsthesia for many hours without the aid of a special assistant. The peristalsis usually removes the surplus of gas from the intestines, if the gas is not generated too rapidly, and a moderate meteorism can easily be removed by gentle massage. The absorption seems to take place in the rectum, at least there was no ether present in the small intestines in cases of complete anæsthesia from a moderate generation of ether vapor. In dogs between twenty minutes and half an hour is required for a thorough anæsthesia. But during this period there seems to be no danger whatsoever for the animal. An injection of morphine facilitates the result without increasing the danger. The rectal tube should be fastened so as to prevent its expulsion by the peristalsis, and the ether bottle should be kept higher than the rectum, in order to prevent the contamination of the ether by intestinal contents.

Anæsthesia by the rectum has the following advantages: If performed properly it is by far less dangerous to the animal than anæsthesia by inhalation. It requires little attention, and no special assistant. It does away with all the reflexes affecting respiration, heart-beat, and blood-pressure, which are such disturbing elements in anæsthesia by inhalation.

Rectal anæsthesia was suggested by Pirogoff for surgical operations as early as 1849. It did not come into practical use until the beginning of the eighties, when it was tried abroad and in this country. The slow procedure, the tenesmus, and the possibility of meteorism prevented its general use. Some experiments were made on animals, but only for testing its practicability for surgical purposes. So far as I know, no previous attempt to introduce it for laboratory purposes, has been made.

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#### DEMONSTRATION: A SIMPLE METHOD FOR THE REDISTENTION OF THE COLLAPSED LUNG.

By S. J. MELTZER.

THE method mostly employed for the redistention of the collapsed lung (in animals) is the sucking out of the air from the pleural cavity. But any one who has had extensive experience with this method knows how unsatisfactory it is. In many cases a piece of the lung is firmly sucked into the cannula, or if the opening in the chest is

made in the sixth intercostal space, or lower, it often happens that even the diaphragm is sucked into it. With the aid of my pleural cannula I have demonstrated on a rabbit a simple method for the redistention of the collapsed lung, and the re-establishing of negative pressure in the pleural cavity. The protruding nozzle of the cannula is connected with a Müller's valve. Then the hand is placed upon the abdomen, and the stomach and the liver are pressed into the thorax while the trachea is being compressed. As the air of the compressed, non-collapsed lung cannot escape through the trachea, it enters into the collapsed lung and distends it. By this distention, and by the pressure from below, the air is driven out of the perforated pleural cavity, while the valve prevents the entrance of air.

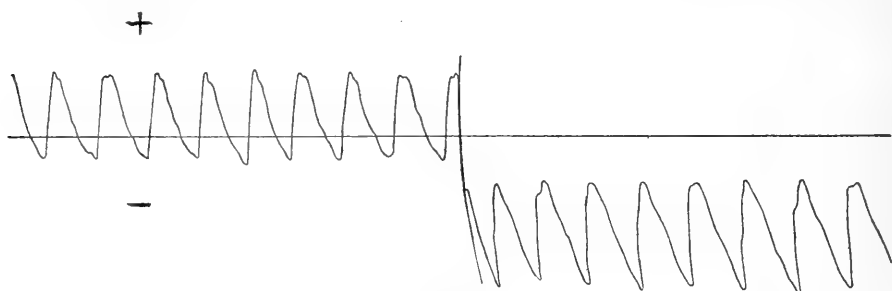


FIGURE 1.

When now the stopcock of the cannula is closed, the tube leading to the valve removed, and the nozzle connected with a manometer, the latter immediately shows a negative pressure. In the experiment illustrated by Fig. 1 the nozzle of the cannula was connected with a Marey's tambour. The straight line was drawn under normal atmospheric pressure; all above the line is at positive, and all below at negative pressure. The undulations at the left were obtained from the pleural cavity while it contained air. The expiration was always positive. Then the lung was distended, and the air driven out by the method described above, and the cannula again connected with a Marey's tambour. Both expiration and inspiration were now below the line of the atmospheric pressure.

ON CERTAIN CHARACTERISTICS OF THE PRESSURE  
SENSATIONS OF THE HUMAN SKIN.

BY G. P. CLARK.

VON FREY has shown that the effectiveness of non-painful mechanical stimuli, in exciting the so-called sense of pressure of the human skin, depends upon certain factors in addition to the strength of the stimulus, namely, the rapidity of its application, the size of the surface to which it is applied, and the locality of the skin stimulated. He determines the value of the physiological factor, the so-called "pressure-points," of any skin surface, by the use of test-hairs (*Reizhaare*), the pressure of which is calculated from careful measurements of the applied surface and the power, *i. e.*, the weight which each can balance on the scales. Finding that test-hairs of greater surface and power are more effective physiologically than those of smaller surface and power, but of the same hydrostatic pressure, he assumes that the nerve organs concerned in the pressure sense are situated somewhat deeply in the skin. An object of the research here reported was to determine whether the same organs in the skin, which have been shown to be called into action by the deformation caused by pressure (*Druck*), are also excited by that caused by traction (*Zug*), or whether other organs are concerned. The movements of the structures underneath the skin may evidently change the tissue pressure of the skin, either increasing or diminishing it, according to the kind of movement and the relation of the skin to the part moved. Changes of pressure, corresponding to those of pressure or traction from without upon the surface of the skin, may thus arise. Tests were made upon very small (0.3 to 0.5 mm<sup>2</sup>.) and large (10 to 50 mm<sup>2</sup>.) surfaces on the left wrist and thumb, and with momentary and continued stimuli of different strengths. The stimuli were applied by means of a double-arm wooden lever in equilibrium, the end of one arm being connected by a very light straw with the surface of the skin to be stimulated, the end of the straw, or of a cork disc, which was slipped on to it when increase of surface was desired, being glued to the skin. The forearm of the person upon whom the tests were made was held in a plaster of Paris mould. Weighting or striking the arm of the lever between its axis and the skin served to produce pressure; weighting or striking the opposite arm produced traction. It was found that the so-called

"pressure-points" most sensitive to pressure are also most and equally sensitive to traction; that with very small surfaces ( $0.3 \text{ mm}^2$ .) there is inability to distinguish between pressure and traction, even with strong and continued stimuli; and that fatigue produced by a strong continued pressure stimulus is fatigue for effects of subsequent momentary traction as well as pressure stimuli. With large surfaces ( $50 \text{ mm}^2$ .) it was found that with momentary stimuli, even of marked strength, there is inability to distinguish between pressure and traction, and that continued stimuli may be of insufficient strength to enable one to distinguish those of pressure from those of traction.

Collectively the tests showed that ability to distinguish between pressure and traction depends upon the size of the surface stimulated, the duration of the stimulus, and the strength of the stimulus; that it is not an inherent quality of the impulse excited in the nerve organs of the skin by the changes of pressure. It having been found that the points most sensitive to pressure are also most sensitive to traction; that simple sensations of deformation are provoked by simple stimuli in either direction; that fatigue for pressure is also fatigue for traction; and that the factors, strength of stimulus, rapidity of application, size of surface to which the stimulus is applied, and locality of skin stimulated are of the same value in the effectiveness of traction stimuli as they have previously been found to be in that of pressure stimuli; — it is assumed that the same nerve organs in the skin are excited by both kinds of stimuli.

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## THE MOVEMENT OF FOOD IN DEGLUTITION.

By A. MOSER AND W. B. CANNON.

[Reported for H. P. BOWDITCH by W. T. PORTER.]

By mixing subnitrate of bismuth with the bolus, the passage of the food along the œsophagus can be seen with the Roentgen rays. In the cat, solid and mushy boluses are carried down by peristalsis, the descent being more rapid in the upper thoracic region than in the neck or below the level of the heart. Liquids descend faster than solids or soft solids as far as the level of the heart, but often remain there for several minutes before a peristaltic wave pushes them into the stomach. In man, solids and soft solids are likewise forced down the œsophagus by peristalsis.

THE MOVEMENTS OF THE STOMACH, STUDIED BY  
MEANS OF THE ROENTGEN RAYS.

By W. B. CANNON.

[Reported for H. P. BOWDITCH by W. T. PORTER.]

THE conclusions reached in this investigation are as follows:  
(1) By mixing a harmless powder, subnitrate of bismuth, with the food, the movements of the stomach can be seen by means of the Roentgen rays.

(2) The stomach consists of two physiologically distinct parts: the pyloric part and the fundus: over the pyloric part, while food is present, constriction-waves are seen continually coursing towards the pylorus; the fundus is an active reservoir for the food, and squeezes out its contents gradually into the pyloric part.

(3) The stomach is emptied by the formation, between the fundus and the antrum, of a tube along which constrictions pass. The contents of the fundus are pressed into the tube, and the tube and antrum are slowly cleared of food by the waves of constriction.

(4) The food in the fundus is not moved by peristalsis, and consequently it is not mixed with the gastric juice; it can therefore undergo salivary digestion in this region for a considerable period without being disturbed. The food in the pyloric portion is first pushed forward by the running wave, and then by pressure of the stomach wall is returned through the ring of constriction; thus the food is thoroughly mixed with gastric juice and is forced by an oscillating progress to the pylorus.

(5) The pylorus does not open at the approach of every wave, but only at irregular intervals. The arrival of a hard morsel causes the sphincter to close tightly, thus materially interfering with the passage of the already liquified food.

(6) Solid food remains in the antrum to be rubbed by the constrictions until triturated, or to be softened by the gastric juice, or later it may be forced into the intestine in the solid state.

(7) The constriction-waves have, therefore, three functions: the mixing, trituration, and expulsion of the food.

(8) At the beginning of the act of vomiting the gastric cavity is separated into two parts by a constriction at the beginning of the antrum; the cardiac portion is relaxed and the spasmodic contractions of the abdominal muscles force the food through the opened cardia into the œsophagus.

(9) The stomach movements are inhibited whenever the animal shows signs of anxiety, rage, or distress.

The full paper will be published in the next number of this Journal.

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## NEW EXPERIMENTS ON THE MAMMALIAN HEART.

BY W. T. PORTER.

I. The recovery of the whole heart from fibrillary contractions; see this Journal, vol. i, page 71.

II. The effect of the beat of the heart upon the flow of blood through the walls of the heart; see this Journal, vol. i, page 145.

III. A method for the study of the blood-currents at the root of the aorta.

A small cylinder, covered with lead foil, and of the same specific gravity as the blood, is fastened by a very short thread to the end of a probe and passed through the carotid artery and aorta to a position just above the semilunar valves. The movements of the cylinder are those of an equal mass of blood. They may be watched with the Roentgen rays after the removal of the ribs.

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## THE EFFECT OF INANITION ON THE STRUCTURE OF NERVE CELLS.

BY F. W. BARROWS.

THE researches to be described were undertaken in order to find out by what structural alterations, if any, a starved nerve cell may be distinguished from one that is well nourished.

In each of three experiments, three rats of the same sex, and similar in weight and general condition, were kept in mechanical cages side by side. Kymograph records gave a continuous history of the activities of each animal during the experiments, together with the temperature and atmospheric pressure for each moment of time. A study of these records shows that fatigue as well as starvation is a strong factor in producing the effects noted. Upon the death of the famished rat, the control rat was weighed and killed. The tissues of the famished and control animals selected for comparison were treated together in the manner described by Dr. Hodge in his work on Fatigue. By this method, the tissues of the normal and famished



animals received exactly the same treatment from the moment of dissection until they were mounted together on the same slide. Microscopical comparison and measurement of normal and famished nerve cells from the occipital cortex, spinal ganglia, and cord, shows: —

(1) A decided shrinkage in size of the cells and nuclei in the famished animals, averaging about 20 per cent, and a still greater shrinkage in the nucleoli.

(2) An evident exhaustion of the substance of famished cells, as shown by their faint staining with osmic acid and the notable absence of nuclei and nucleoli. The protoplasm of these cells shows a very fine vacuolation, not so marked as that described by Rosenbach for starving animals, and by Hodge for extreme fatigue. In the brains of famished rats the pericellular lymph spaces are considerably enlarged.

THE COMPOSITION AND NUTRITIVE VALUE OF SOME EDIBLE AMERICAN FUNGI.

By LAFAYETTE B. MENDEL.

See this Journal, vol. i, p. 225.

SOME EXPERIMENTS ON THE EXCRETION OF KYNURENIC ACID. By L. B. MENDEL.

DEMONSTRATION: A NEW PLEURAL CANNULA *IN SITU*. By S. J. MELTZER.

A description of the cannula will be published in this Journal.

DEMONSTRATION: THE NUTRITION OF THE MAMMALIAN HEART THROUGH THE VESSELS OF THEBESIIUS. By W. T. PORTER (for F. H. PRATT).

See this Journal, vol. i, p. 86.

A BASIS FOR A THEORY OF COLOR VISION. By W. PATTEN.

INFLUENCE OF ALCOHOL UPON THE YOUNG IN DOGS AND UPON THE SEVERITY OF AN ATTACK OF DISTEMPER. By C. F. HODGE.

Read by title.

INFLUENCE OF ALCOHOL UPON VOLUNTARY MUSCULAR POWER IN CONDITIONS OF FATIGUE. By C. F. HODGE.

Read by title.

THE INFLUENCE OF BILE AND BILE SALTS ON PANCREATIC PROTEOLYSIS.

By R. H. CHITTENDEN.

Read by title. This paper will appear in the next issue of this Journal.

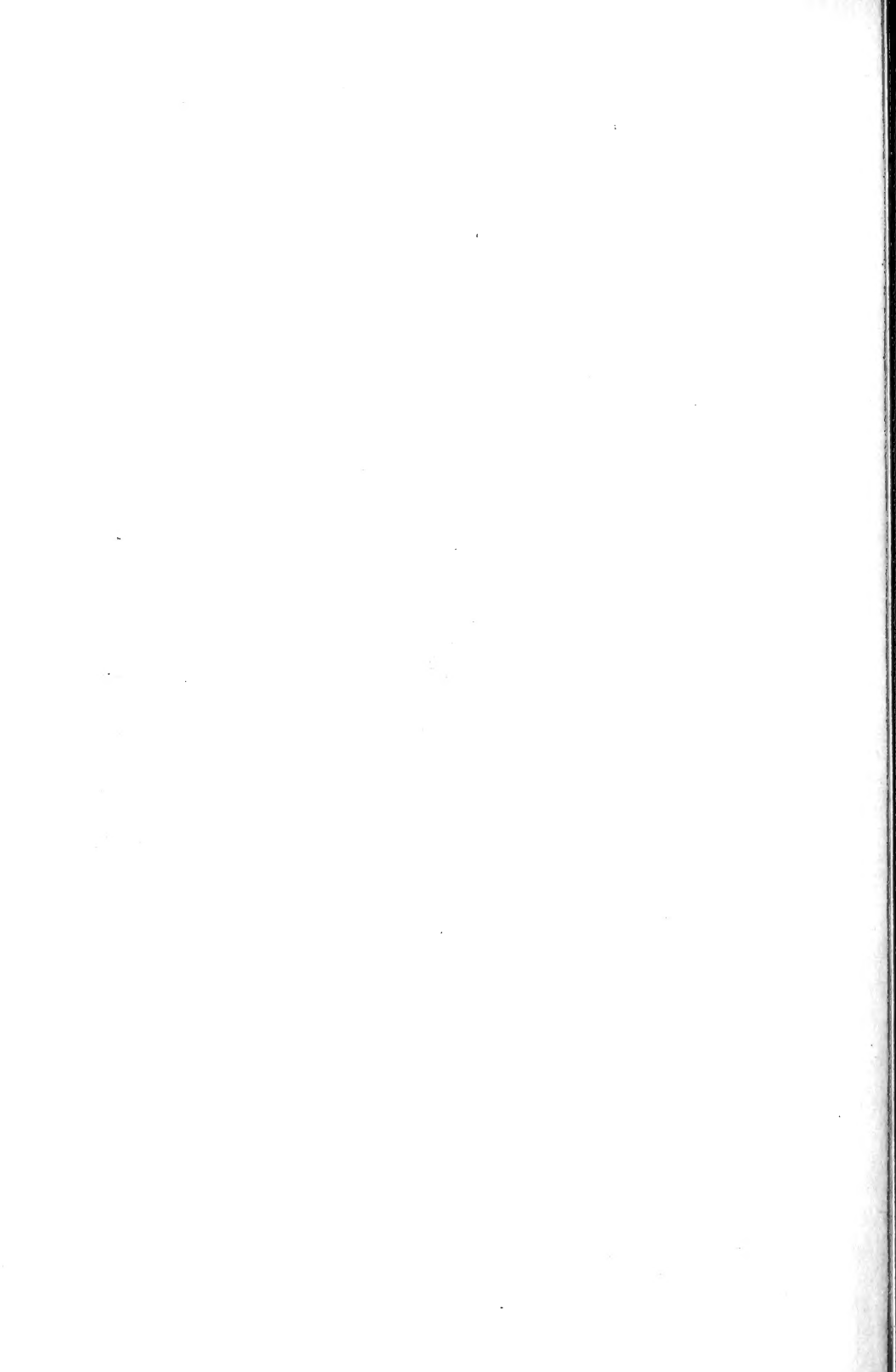
THE BIOLOGICAL PROBLEMS OF TO-DAY: PHYSIOLOGY. By J. LOEB.

665









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